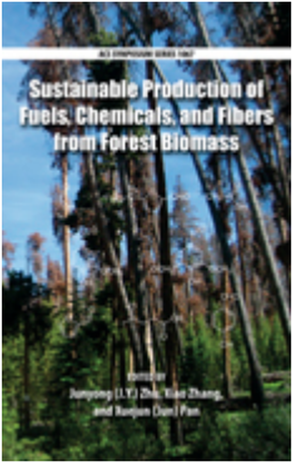

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Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass

EDITED BY
Junyong (J.Y.) Zhu, Xiao Zhang,
and Xuejun (Jun) Pan

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Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass

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Foreword

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Preface

Wood has always been—and continues to be—an important natural resource. Millions of tons of wood and forest biomass are harvested each year and converted to construction materials, commodity products, specialty chemicals, and fuel. We may have long taken for granted this invaluable natural resource bestowed on us through photosynthesis, partly because we have indulged in petroleum-based products and energy for over a century. Concerns over depletion of fossil fuel and global climate change drive us to search for alternative sources of energy that are sustainable and have less environmental impacts than petroleum. Although we have a number of potential options, forest biomass stands out because of (1) a vibrant forest products industry that is equipped with efficient infrastructures, logistics, and highly skilled human capital and (2) the potential for replacing the entire spectrum of petroleum-based products and chemicals with renewable materials and chemicals from forest biomass.

We realized the huge challenges to developing and commercializing new technologies to the scale of several thousand tons of biomass per day (which may take decades) and the significant benefits—and potential short cut—of aligning with century-old industrial giants, such as the forest products industry, with mature and efficient production capabilities. Therefore, we produced this book to offer a perspective on transforming the technologies, infrastructures, and knowledge that are part of forest products manufacturing processes to help establish a forest biorefinery industry for sustainable production of energy, chemicals, and products. The forest products industry has a wealth of experience and history of innovation in producing a variety of biomaterials, such as engineered wood products, pulp and paper, cellulose derivatives, textile materials, and specialty chemicals. We believe that it would be imprudent to overlook the immense opportunity for developing next-generation bioproducts, chemicals, and materials from forest biomass. Our objectives for producing this book are to exploring this opportunity.

We acknowledge the many technical and economic challenges to be overcome before a truly sustainable biomass-based biorefinery industry can be established. Nature constructs forest biomass as a structural material with strong recalcitrance to physical, chemical, and microbial deconstruction. As a result, bioconversion of forest biomass to readily processible molecules for biofuel, bioproducts, and chemicals is inevitably a difficult technological and economic challenge. Nevertheless, we present a collection of state-of-the-art developments in a number of forest biomass conversion research areas in this book. We hope that the information will help to attract more interest in exploring the vast potential of utilizing forest biomass as a resource to strengthen the sustainability of our society.

We are deeply indebted to all the chapter authors for their hard work and timely contributions. We are grateful to the American Chemical Society for providing this opportunity, and we sincerely thank all the reviewers for their valuable contributions to the structure of this book and the quality of each chapter.

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Chapter 1

Forest Biomass Sustainability and Availability

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This chapter provides a synthesis of information on potential supply of forest biomass given needs for sustainable development of forestry. Sustainability includes maintenance of water supply, biodiversity, and carbon storage as well as timber products, community development, and recreation. Biomass removals can reduce fire hazard and insect and disease attack, restore forest composition and structure, enhance forest growth, provide revenue for treatments and communities, and offset greenhouse gas emissions. Biological limitations vary by forest condition, ownership, and how stands are regenerated. Limitations maintain water supply, soil nutrients, and biodiversity. There are economic limitations because costs for removals may exceed revenue. One analysis suggests U.S. forest-based biomass supply could be 45 million dry tons per year or more, depending on biomass price. Social targets and limitations are given in federal and state legislation. These include a federal cellulosic fuel target with biomass source restrictions, state-level renewable energy portfolio standards, and state-level forest practice guidelines. Understanding of biological and economic limitations and benefits is developing, particularly at local levels. Social targets and limitations could change. Increases in fossil fuel prices would accelerate efforts to develop understanding of biological limitations and could result in changes to social and economic targets and limitations.

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Introduction

To assess availability and sustainable use of forest biomass for the United States, we suggest accepting the "Brundtland report" (*1*) view of sustainable development. This report defines sustainability in relation to satisfying human needs though sustainable development: *Humanity has the ability to make development sustainable—to ensure that it meets the needs of the present without compromising the ability of future generations to meet their own needs. The concept of sustainable development does imply limits—not absolute limits but limitations imposed by the present state of technology and social organization on environmental resources and by the ability of the biosphere to absorb the effects of human activities.* This definition indicates that sustainability needs to account for (1) human needs, current and future, (2) limitations in meeting these needs based on technological capabilities, social organzation, and environmental resources, and (3) the ability of the biosphere to absorb the effects of human activities.

We also suggest that sustainable development of forest biomass needs to be part of a broader sustainable development of forestry that seeks to meet a wide range of social, product, and environmental needs for people. Specifically the sustainable development of forest biomass must consider how to accommodate sustainable development of forest timber products, forest-based recreation, forest non-timber products, forest-based community development, and forest environmental services such as biodiversity and carbon storage (*2*).

Efforts to establish sustainable levels of forest biomass use need to consider the potential biological supply and a range of environmental, economic, and social limitations. The sustainable development of biomass, although subject to many limitations, also potentially provides benefits that can promote sustainable development of forestry. This chapter provides synthesis of current understanding about the factors that will determine the sustainable levels of biomass supply. First, we provide three example analyses that estimate potential sustainable supply given current understanding of economic and environmental constraints. The first example estimates of potential biomass supply are driven by physical and biological limitations. The second example estimates add economic limitations and estimate supply available at different prices at forest roadside or mill gate. The third example estimates add limitations on biomass supply due to competition with agricultural biomass and limitations of transportation and technology to produce biofuels in specific locations across the western United States. In progressing through the examples, there is a progression of added constraints that will determine supply of forest biomass. Each example has similar but not identical biological limitations on supply. Second, we discuss environmental benefits and limitations associated with forest biomass supply. Third, we discuss the social (institutional) support and limitations on biomass supply—which may not be included in the economic/environmental estimates—including current and emerging regulations and policy concerning renewable energy, and forest practice guidelines.

Our second and third biomass supply estimation examples focus on estimating biomass supply for biofuels production. In addition to the factors that determine biomass supply in these examples, the amount of wood biomass actually available in a location for a particular user will be influenced by competition for biomass from electric power plants, thermal energy producers, biochemical producers, and users of pulpwood to make paper and wood panels. Even though our examples focus on estimating biomass for biofuels production, they are in fact estimates of supply available for all wood biomass users.

Table I shows key estimation results for the three examples and the key drivers used to make the estimates.

Table I. Comparison of three estimates of forest-based biomass supply

Study	*Key Estimate*	*Key drivers of biomass supply estimates*
"Billion Ton Supply" Study	Nationwide estimate—137 million odt/year of forest-based biomass supply physically available in the near term	• Physical and biological limits on logging residue removal • Need for and biological limits on forest health thinnings
BRDI Feedstock Study	Nationwide estimate—45 million odt/year of forest-based biomass supply available for a cost of about $44/odt at forest roadside or mill gate in the near term	• Cost to harvest and chip progressively larger amounts of logging residue or biomass from forest thinnings • Limitations on amounts logging residue or thinnings obtained due to required co-production of sawlogs/pulpwood
WGA Biofuel Plant Siting Study	Western U.S. estimate—11 million odt of forest-based biomass supply out a total supply of 130 million odt of all forest and agricultural feedstock; total biomass would make 11 billion gallons of biofuels at a demand price of $2.40 per gallon gasoline equivalent	• See drivers for BRDI Feedstock Report • Spatial distribution of biomass in relation to candidate locations for biofuels plants • Transportation networks and costs • Capital and operating costs for conversion technologies • Offered price of biofuels at wholesale fuel terminals

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Potential Biological Supply from Forest-Based Biomass Resources

A general definition of forest biomass is all forest plant and forest-plant-derived materials. We limit forest biomass to parts of trees on, or derived from, forest land. This definition includes all trees on two categories of forest land—timberland and other forest land. Timberland can grow 20 cubic feet per acre per year on the main stem of trees, whereas other forest land (such as pinyon-juniper forest in the western United States) produces less. It also includes wood and bark residues generated at primary mills (sawmills, panel mills, or pulp mills) or secondary mills (e.g., flooring, furniture).

We do not discuss wood that may come from growing short-rotation woody crops (SRWCs) on agricultural land because this is covered in another chapter. Our focus is on the level of sustainable supply from forest-based sources. Factors that determine biological potential for SRWCs, economic limitations, and social limitations differ from factors for forest-based sources. Sustainable supply of wood from SRWCs is influenced by many factors, including rates of improvement in genetic stock and growth and the amount of shift of agricultural land to SRWC and associated decrease in food production that is acceptable. The total amount of wood biomass available for biochemicals and bioenergy will be the total available from SRWC and forest-based sources.

Estimates of wood biomass supply from forest-based sources often focus on amounts not currently used for lumber, panels, or paper. Specifically, unused forest biomass resources can include (1) logging residue left after conventional harvesting operations, (2) "other removals" from land clearing or pre-commercial thinning operations, (3) fuel treatment thinnings to reduce fire hazard or insect and disease damage, (4) unused mill residue from primary wood processing mills, and (5) unused mill residue from secondary processing mills. It is sometimes the convention to include several other sources as forest-based sources, including urban wood residue, construction and demolition waste, and tree trimmings, and we include them for that reason. Physical and biological availability from sources (1) to (5) is driven by different factors. Logging residue availability is driven by (co-produced with) harvest of sawlogs and pulpwood and limited by need to leave nutrients on logging sites. In a similar way sources (2), (4), and (5) are co-produced with other activities. Forest treatment thinnings are driven by forest restoration objectives and programs. Each of these estimates excludes use of biomass supply that would require diverting wood from its current uses. Diverted sources include (1) pulpwood, (2) sawlogs (at a very high cost), (3) fuelwood (used primarily for home heating), (4) currently used wood residue from forest products mills, and (5) black liquor from pulp mills. Diverted biomass sources are discussed separately.

Our first estimation example is from the USDA/DOE "Billion Ton Supply" report (*3*), which estimated sustainable biomass use to determine if U.S. land resources can produce biomass sufficient to displace 30 percent of the country's 2005 petroleum consumption, or about 1 billion dry tons of biomass per year. The report estimated potential supply of 1.3 billion dry tons of biomass by the mid-21st century and that forest biomass may provide up to 368 billion dry tons. However, for forest sources, if we exclude amounts currently used and amounts based on assumed growth of the forest products industry (e.g., more logging residue and mill residue) then additional near-term forest biomass potential is 137 million dry tons (Tables II and I). If we take into account amounts of biomass generated with increased production by the forest products industry, then biomass potential is 225 million dry tons.

An increase of forest biomass supply of 137 million dry tons, or with industry growth to 225 million dry tons, would be an increase over 2006 U.S. wood harvest (225 million dry tons) (*3*, *4*) by 60–100 percent. The biomass increase with industry growth assumes all increases in fuelwood, wood residue at mills, and black liquor would be available for new bioenergy and biochemicals. There is a good case for the sustainability of these additional biomass levels based on

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limitations built into the estimates. These include (1) current conventional harvest and projected future harvest that produce logging residue are well below forest growth, (2) it is likely that leaving behind 35 percent of logging residues would provide nutrients and habitat needed to sustain forest ecosystems and wildlife, (3) additional biomass from fuel treatments to reduce fire hazard is based on estimated possible thinning removals of wood from currently overstocked forest stands and would be removed over 30 years, after which additional growth could allow additional thinnings, and (4) currently unused mill residue is excluded from supply. Note that these estimates, by excluding diversion of currently used biomass (pulpwood, mill residue, fuelwood, pulp liquor) would also allow for sustaining a growing forest product industry to meet needs for increasing domestic consumption of wood and paper products.

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Table II. Potentially available forest biomass for the United States from the "Billion Ton Supply" report (10^6 dry tons)

	Unused	*Existing use*	*Growth*	*Total*
Logging residue	**32**	—	**15**	**47**
Other removal residue	9	—	8	17
Fuel treatments (timberland)	49	—	—	49
Fuel treatments (other forest land)	11	—	—	11
Fuelwood	—	35	16	51
Wood residue (forest products)	8	46	16	70
Pulping liquor (forest products)	—	52	22	74
Urban wood residue	28	8	11	47
Total	137	141	88	366

If the constraint is removed to avoid diverting currently used mill residue, fuelwood, or pulp liquor, then some fraction of the exiting biomass use of 141 million dry tons (includes black liquor) could be diverted to new bioenergy or biochemical production.

The "Billion Ton Supply" report does not include potential biomass supply from either (1) additional pulpwood harvest or (2) diversion of current pulpwood harvest from pulp mills and composite panel mills. The amount of U.S. pulpwood harvest in 2008 was about 74 million dry tons, down from about 93 million dry tons in 1998 (*5*, *6*). Therefore, pulpwood harvest could be increased by at least the difference between the 1998 and 2008 harvest levels, or 19 million dry tons. Such increased pulpwood harvest would also generate logging residue that could be partially removed.

Using a conversion factor of 80 gallons of ethanol per dry ton of biomass or the potential sustainable ethanol production using additional biomass sources—without industry growth and without substantial diversion—wood from current uses could be ((137 + 19) × 80) = 12 billion gallons. These estimates of *potential* biologically sustainable supply could be increased with diversion of wood from current sources and additional pulpwood harvest. However, total *available* supply will also be limited by economic (cost) and social (e.g., regulatory) constraints.

Example Estimates of Supply Given Economic and Environmental Constraints

A number of national and regional studies have estimated forest biomass supply considering economic synergies and limitations. In addition to the physical and biological limitations identified in the "Billion Ton Supply" report, there are economic limitations on total supply that include (1) assumptions about markets or programs that will provide forest biomass, (2) costs to purchase, harvest, and chip biomass for roadside pickup, and (3) cost to transport biomass to using facilities. One determinant of the cost to harvest is the expected level of special equipment owned by harvesting companies to efficiently remove biomass, chip, and transport wood biomass. The limitations on supply to a particular user in a region will also include competition for biomass from other users—for biofuels, bioelectricity, thermal energy, and pulpwood use for pulp or panels. These economic limitations are all on the supply side—the amount of biomass that may be supplied at a given price at a plant gate. The amount of biomass a plant can consume in a particular location depends on the price it can pay—a limitation on the demand side. The amount the plant can pay will be determined by other non-feedstock costs of production and by the price of fossil fuel that could be used as an alternative to biomass. Increases in price for fossil fuels will be a key factor in determining the speed of development of bioenergy plants (*7*).

Our second and third examples of estimates of biomass supply include economic constraints. The first focuses on estimates of forest biomass or mill residue available at increasing roadside or mill gate costs. The second adds limitations on biomass supply due to competition with agricultural biomass and limitations of transportation and technology to produce biofuels in specific locations across the Western United States.

Economic Estimates of National Forest Biomass Supply for Biofuels Including Economic Constraints

An analysis commissioned by the U.S. Federal Biomass Research and Development Initiative (BRDI) (*8*) shows how assumptions about operation of wood products markets and allowable cost levels for forest-based biomass and competition with agricultural biomass supplies will result in limited amounts of biomass being available for biofuels and chemicals production.

The BRDI analysis determined the agricultural and forest-based biomass sources and cost levels required to produce 21 billion gallons per year of advanced biofuels (e.g., cellulosic ethanol or biodiesel) (*8*). The 21-billion-gallon production target for advanced biofuels in 2022 is specified by the 2007 Energy

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Independence and Security Act. The analysis assumed 1 billion gallons of the total would be biodiesel from soybeans. The remaining 20 billion gallons would be provided using a combination of agricultural feedstocks, forest-based feedstocks, and imported biofuels.

Note that the BRDI analysis assumed that all forest-based and agricultural biomass would be available for biofuels. In reality, these biomass sources could also be used for increased electric power production or increased heat energy production, so not all the amounts estimated by the BRDI report would necessarily be available solely for biofuels. If competition with electric power producers for biomass were included in the analysis, the amount of biomass available for biofuels at a given price would depend on biomass demand by electric power producers.

The first step in the BRDI evaluation was to estimate quantities of agricultural and forest-based biomass that would be provided at forest roadside or farm gate at progressively higher costs. To provide enough forest and agricultural biomass feedstock to produce 20 billion gallons, it was determined that the roadside/farm gate cost would need to be about $44 per ovendry ton (odt). At this price level, about 45 million odt of forest-based biomass would be provided that could make 4 billion gallons of ethanol. Agricultural biomass feedstocks or imports would be used to provide the other 16 billion gallons of biofuels. The study did not estimate the cost to provide biomass delivered to biofuels plants. The delivered cost would depend on local factors, including spatial distribution of the biomass, transportation infrastructure, and biofuels plant size (these factors are included in our next example).

The estimated supply of forest-based biomass of 45 million odt at $44/odt (Table I) is substantially less than the potential supply identified by the "Billion Ton Supply" report—where the estimate of current potential was 137 million odt/year and the estimate of potential with an increase in harvest of traditional products was 225 million odt/year. There are two key reasons why the BRDI estimate is much lower. First, the "Billion Ton Supply" report assumed that it may be possible to collect biomass from logging residue at conventional logging sites and, in addition, conduct thinning operations on separate forest land to reduce fire hazard and insect and disease damage. However, the BRDI analysis needed to be explicit about the markets or programs that would provide biomass for biofuels. To obtain logging residue requires integrated harvesting operations providing both sawlogs/pulpwood plus biomass. To obtain biomass from separate thinning operations requires that either they be integrated harvesting operations that provide both sawlogs/pulpwood plus biomass, or that there would be quite expensive subsidized operations that would remove only small trees and biomass suitable for biofuels or bioenergy. For the BRDI report, it was assumed that thinning operations would integrate harvesting operations where sawlogs/pulpwood are harvested along with biomass. The estimated amount of biomass provided in total by logging and thinning operations is limited by the expected demand for sawlogs and pulpwood in each region.

The second main factor limiting supply is the cost to provide forest-based biomass at roadside. Amounts of biomass from integrated forest harvesting operations have a stumpage cost (cost of biomass resting in the forest) plus cost to harvest and chip at roadside. While logging residue currently has a stumpage

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cost about \$3 per odt, the cost increases toward pulpwood stumpage prices (e.g., \$15–\$25 per odt) as removals increase. The price would increase as available residue becomes more scarce. Harvesting and chipping costs increase as more stands are harvested that have a lower density of trees or greater haul distance to roads.

Table III indicates the effect of the integrated harvesting and cost restrictions on availability of forest-based supply sources. Logging residue and forest thinnings could provide 40 and 21 million odt/year, respectively, but after imposing a limit that (1) integrated logging to provide only as much sawlogs and pulpwood as currently demanded and (2) a roadside price of \$44/odt, then supply from these sources is limited to 20 and 11 million odt, respectively. Higher prices for logging residue and thinnings bring higher supply because it allows access to biomass with higher harvest (as determined by simulation of thinnings and harvest operations) and stumpage costs. Other removal residues (from land clearing and pre-commercial thinnings) are limited to half the total potential—6 million versus 12 million odt. Urban wood residue is limited to about 10 percent of the total estimated residue generated of 28 million odt. The upper limit of supply, at any price, is judged by half the amount generated. Conventionally sourced wood (pulpwood) is estimated to contribute about 4 million odt to meet the goal to produce 20 billion gallons of biofuel, whereas the potential at higher prices is at least 15 million odt/year.

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Table III. Forest biomass available at about \$44 per ovendry ton and at cost over \$100 per odt for the United States from the BRDI feedstock report (10^6 dry tons)

	Supply at \$44/odt	*Upper limit on supply >\$100/odt*
Logging residue	20	40
Other removal residue	6	12
Fuel treatments (timberland)	11	21
Fuel treatments (other forest land)	0	0
Wood residue (forest products)	1	1
Urban wood residue	3	14
Conventionally sourced wood (pulpwood)	4	15
Total	45	103

As prices increase above \$44/odt, supply would increase above 45 million odt as more is supplied particularly from conventionally sourced wood and urban wood waste, which could add at least 30 million odt (Table III). With increased price, the increased supply would vary notably by source. Supply of biomass from logging residue and thinnings may not increase much with price to the extent that supply is limited by sawlog and pulpwood harvest levels. Supply from new or diverted pulpwood harvest can continue to increase with price as limited by owner willingness to sell and harvest costs. Supply from urban sources will increase in price as limited by wood waste amount and collection and processing costs. The amounts from logging residue and thinnings could most readily increase with increased levels of sawlog and pulpwood harvest, which would be required to get the higher levels of logging residue and thinning supply shown in Table III at prices over $100/odt. In projections of harvest made in 2005, conventional sawlog and pulpwood harvest was projected to increase by 29 percent over the 2006 level by 2030 (*9*). A revision to these projections would take into account the decrease in recent economic activity due to the recession and the impact of any increased demand for pulpwood use for energy.

The estimates prepared for the BRDI report exclude supply of forest biomass from federal lands in recognition that the Energy Independence and Security Act of 2007 excludes use from most federal land to meet biofuels production targets. However, the analysis does not apply the EISA 2007 restriction to use only forest biomass from planted forests. Such a restriction would substantially reduce supply (see discussion on social limitations below). There is currently no federal legislation promoting electric power production or thermal power production that restricts biomass supply to come from planted acres.

The estimate of 15 million dry tons for pulpwood supply from the BRDI report does not include the effect of increasing supply due to increases in forest inventory over time nor the supply of biomass from pulpwood diverted from current pulp or panel production. A study by Abt et al. (*10*) estimated that over half of 30 million dry tons of wood biomass needed to provide 10 percent of the fuel for coal electric power plants in the South would come from additional pulpwood harvest or pulpwood diverted from current supply to pulp and panel mills. In this case, half or more of biomass supply would come from pulpwood sources as opposed to logging residue sources. Compared to the BRDI study, this study for the South estimates greater proportion of supply from pulpwood sources as opposed to logging residue sources.

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Example Estimates of Western Biofuels Supply Including Competition with Agricultural Sources and Limitations of Infrastructure and Technology

Our third example is from an analysis commissioned by the Western Governors Association (WGA) and shows how the use of forest-based biomass will be limited not only generally by wood product markets and allowable cost levels (as for the BRDI analysis) but also by amounts close to specific geographic locations. That is, the amount of forest-based biomass available for a specific plant will be limited by the amount of material available at a certain cost within a certain transportation radius. There will be a tradeoff between the higher cost of obtaining greater supply at greater distances and the lower capital cost of a larger capacity biofuels plant.

The WGA study determined the amount of biofuels that could be produced in the western United States using forest-based and agricultural resources for given prices of biofuels at existing fuel terminals. Given an offered price for biofuels at fuel terminals across the West, an optimization model was used to select the optimal location of biofuels plants from candidate locations and simultaneously select the amount of feedstocks to be used from a given local area, the conversion technology to be used, and the capacity of the plant. Optimal locations were ones that provided the highest profit level given the biofuel price offered at terminals. A key finding was that biofuels could provide between 5 and 10 percent of the projected transportation fuel demand in the region with fuel price between $2.40 and $3.00 per gasoline gallon equivalence (gge) at fuel terminals, and exclude costs to deliver fuel to local gas stations and taxes (*11*). At $2.40 per gge 11 million odt of forest biomass would provided in the West or 8.6% out of total feedstock of 130 million odt from all sources. Total biofuels production at $2.40 per gge would be 11.3 billion gallons (Table I).

Figure 1 (from the WGA report) indicates how estimated capital cost to produce a gallon (gasoline equivalent) using Fisher–Tropsch conversion declines with increasing capacity. The WGA study identified the size and technology of plants that would produce biofuels at lowest cost in given candidate locations when considering both the possible size of plant (which determines capital cost per gallon) and aggregate demand for biomass (which determines transport distance and cost per unit of biomass).

The WGA study allowed wood biomass to be converted to liquid fuel using three technologies: hydrolysis and fermentation to ethanol, gasification and Fisher–Tropsch conversion to middle distillates, and upgrading pyrolysis oil to gasoline. Efficiencies to convert wood to biofuels for these processes were assumed to be 90, 42, and 22 gallons per dry ton, respectively. Given these conversion efficiencies and associated costs of production by capacity size, only the first of these technologies was selected by the optimization model to convert wood to biofuels at biofuel prices up to $3 per gge.

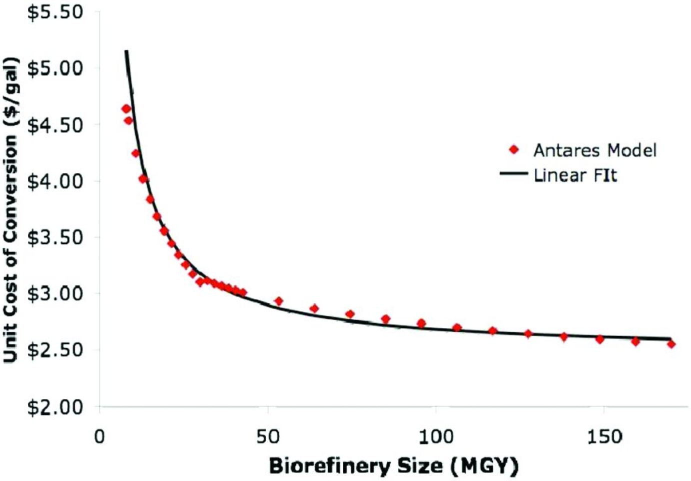

Figure 1. Capital cost per gallon (gasoline equivalent) to produce biofuel by Fisher–Tropsch conversion by biorefinery size. (reproduced with permission from reference (11)).

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

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For a biofuels price of $2.40/gge, the capacity of plants converting wood to ethanol was limited to sizes from 35 to about 100 million gallons per year, depending on the local abundance of forest-based biomass. For example, plants in Northern California at Eureka, Redding, Chico, and Fort Bragg ranged in size from 55 to 98 million gallons (Figure 2). At a conversion efficiency of 90 gallons per dry ton, the annual wood consumption would range from 610,000 to 1,090,000 dry tons per year. The fact that there are four limited-scale lignocellulosic plants chosen by the optimization model also illustrates that in forest areas it may be most profitable to have several smaller scale plants that each obtain biomass from a limited distance to obtain forest biomass, rather than one or two large plants that go a longer distance to obtain biomass.

There are additional caveats to understanding the effect of local forest biomass supply on capacity size. As prices for forest biomass increase above the current price for pulpwood, then pulpwood-size material can be purchased for biofuels, and not just logging residue. When pulpwood may be purchased within an area, then the total biomass available in the area will increase. This would increase the economic viability of constructing a higher capacity biofuels plant. In addition, if prices increase above recent pulpwood prices, then it may be economical to invest in growing short-rotation woody crops within a short distance of a biofuels plant, which could also increase the viability of constructing a higher capacity biofuels plant.

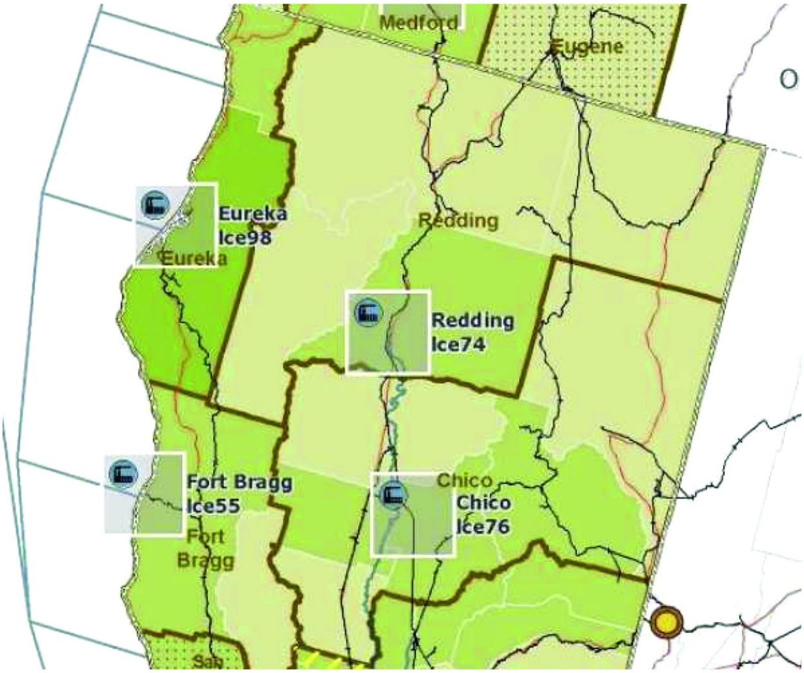

Figure 2. Potential lignocellulosic ethanol plant locations in Northern California. (reproduced with permission from reference (11)).

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Environmental Benefits and Limitations Associated with Forest Biomass Supply

Removal of biomass from forests may have positive or negative effects on site productivity and forest sustainability as determined by what (and how much) is removed and by how biomass is removed (*12*). Sustainability criteria (*13*) include protecting the resource base (*14*, *15*) and maintaining biodiversity (*16*), maintaining carbon storage, and reducing or eliminating net greenhouse gas (GHG) emissions in producing wood-based energy (*17*). Protecting the resource base requires attention to maintaining productivity and avoiding off-site impacts from pesticide or nutrient movement (*18*). Potential negative effects are very specific to management systems and the machinery used to harvest and gather material (*19*). The main environmental concerns are whether high levels of removals and more trafficking by machinery would reduce future productivity by removing too many nutrients, lowering levels of soil organic matter, compacting soil, and increasing soil erosion (*20*). The total effects on carbon balance (*21*) and energy efficiency depend not only on the production and harvesting of biomass but also on the energy it produces, which depends on the efficiency of the conversion technology and products that result as well as offsets of other fuel sources; these aspects require a full life cycle analysis that is beyond the scope of this chapter (*17*).

As noted above, the most likely scenarios for providing biomass are (1) increased residue removals integrated with harvesting timber products (*3*, *19*); (2) removals of biomass to reduce fire hazard (e.g., (*22*)); or (3) harvest on additional areas primarily to provide biomass for bioenergy (*23*). In addition, locally significant amounts of biomass may be available after major disturbances, including wildfire, insect or disease attack, and wind storms. In all cases, the limitations and methods used to remove biomass with lowest impact will be determined by the nature of the existing forest stands. The most salient features of forest stands that will guide limitations and methods are whether they are publicly or privately owned and whether they are planted versus naturally regenerated. Most forest land in the eastern United States is privately owned, and the reverse is true in the West where public ownership predominates (*4*). The main areas of planted forests are the southern pines and the Douglas-fir stands of the Pacific Northwest. The limitations and methods will also be determined by the details of the kinds of wood material that are removed.

Limitations on removals and acceptable removal methods vary in part because of differences in the major management objectives of public and private landowners. Public land tends to be managed for a wider range of resource values than private land. Private landowner objectives tend to include obtaining revenue from timber and other resources. Non-industrial private forest (NIPF) landowners have the most variation in management objectives. Some surveys of NIPF landowners suggest interest in non-timber values can be high and may limit interest in removal of wood for revenue (*24*).

What Material Is Removed

Logging residue is a broad category that includes cut material from conventional harvesting that is not removed (tops, limbs, some green but rotten wood boles) and standing dead and green material that is not harvested because it is unusable (standing dead and rough or rotten stems of merchantable diameter; non-desirable species; and small-diameter stems of commercial species). For planted stands, residues tend to be tops and limbs. For naturally regenerated forests, it is also possible to remove a portion of the non-desirable stems (non-commercial species) and a portion of small-diameter stems of desirable species. The number of small-diameter trees of desirable species that are left standing may have significant effects on the successful regeneration of the stand. Regrowth of the forest with desirable species will be faster if some small trees of desirable species are left standing after harvest. Leaving behind non-desirable trees may hinder successful regeneration of desirable species by competitive exclusion. Currently pulpwood and sawlog harvest from federal forest land is low, so almost all logging residue from integrated harvesting will come from non-federal lands, probably private lands (*3*). Harvest of additional forest areas primarily for biomass will likely be similar to current pre-commercial thinnings or may be similar to current practices to harvest pulpwood with additional removal of some residue.

Biomass from forest treatments to reduce fire hazard is primarily unmerchantable trees and shrubs that have accumulated as a result of altered fire regimes (*25*, *26*). The alteration in fire regimes (frequency of fire) is primarily due to many years of fire suppression. Although there are tens of millions of acres in need of treatments to reduce fire hazard and restore natural fire regimes, available treatment methods are costly and sometimes resisted by the public. Although fuels reduction treatments that provide biomass for bioenergy could overcome some of the financial constraints, the dispersed nature of the biomass imposes significant transportation costs. Financial and logistical concerns place limitation on the number of acres that can be treated per year. The “Billion Ton Supply” report (*3*) assumed that the backlog of needed treatments would occur over 30 years. As a result of assumptions such as no road construction and other operating, the amount of biomass for fire hazard reduction thinnings included in that report as available for bioenergy uses is less than 1 percent of the material that has been identified for fuel treatment removal. The National Forests are projected to furnish about 20 percent of the hazardous fuel removals available for bioenergy from timberlands and from other forest land. Most (73 percent) of the hazardous fuel biomass removals from timberlands and 58 percent of the removals from other forest land will come from privately owned forests. Other publicly owned land makes up the remainder.

Biomass may also come from salvage operations after major disturbances . Wildfire, hurricanes and other wind events, and insect and disease outbreaks, such as bark beetles or exotic organisms, can affect large areas; these disturbances effectively represent unplanned harvests. Salvage logging is conducted to recoup financial value, limit spread of infestation, reduce wildfire risk, or allow for reforestation. While salvage logging is contentious (*27*), such events could

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provide locally significant biomass supply. Additionally, major wind events such as Category 3 and higher hurricanes cause blowdown and breakage that blocks roads, highways, and commercial sites, and the removed material is usually piled and burned or placed in landfills but could be available to produce bioenergy.

How Forest Biomass Is Removed

Methods to remove biomass can be adapted to help obtain the most biomass, meet environmental impact limitations, and lower costs. Conventional harvesting methods are numerous and depend on forest type, regeneration method, sizes of materials to be removed, site conditions such as slope, drainage, trafficability, and costs of operation. In general, direct effects of harvesting are minimized by matching machinery to the site, including use of low ground pressure tires or tracks and minimizing machinery movement on the site. Protecting water resources by delineating buffers around streams and controlling water movement and other site-specific protection practices also minimize direct harvesting impacts. Some form of voluntary Best Management Practices or Forest Practices regulations (discussed below) have been adopted in all states to protect water and other resources, and many address these issues (*28*). Integrating biomass removals with conventional harvesting may use standard harvesting equipment (for example, small-diameter stems or unmerchantable stems may be felled and transported with merchantable stems to a landing or roadside where they are separated and transported).

Unconventional biomass removal methods are being developed to minimize handling and transportation costs of tops, branches, and limbs (*29*). One method gathers, compresses, and bundles this material into a form resembling a standard log, which can then be transported by conventional methods. Besides bundling machinery, in-woods chippers and masticators are available for fuel reduction treatments, particularly in the wildland–urban interface (*30*). In Southern Pine plantations, adding a small chipper to conventional harvesting appears to work better on clearcut sites than in partial harvests (*31*). Specialized machines have been developed in Scandinavia to remove greater forest biomass by lifting stumps and root systems from the soil (*32*). While not currently in general use, they have the potential to increase biomass removal by 25 percent or more of the aboveground removal. If biomass removals are conducted as separate operations and not integrated with conventional harvests, such as mechanical fire hazard reduction treatments, they represent an additional entry into a stand and may be contaminated with more soil and rock and have more or different impact than integrated operations. Additionally, biomass produced may be contaminated with more soil and rock than is typical of conventional harvests.

Effects of Biomass Removals

Potential negative effects of biomass removals require limitations and are primarily a function of how much and what is removed (nutrients, deadwood) and ground disturbance (soil compaction, erosion, and impacts on regeneration and ground layer diversity). Biomass removals may also have positive effects on forest health and assist to restore degraded ecosystems. Potential negative effects

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are very specific to management systems and the machinery used to harvest and gather material. The main environmental concerns relate to impacts on soil's physical and chemical properties, particularly maintenance of fertility. Fertility reduction is a concern because nutrient concentrations are higher in leaves, twigs, and small branches than in bolewood, and there is an argument that over time more nutrients would be removed with entire removal of tops and branches than could be replenished from natural sources. Similar concerns about removals from planted forests have been addressed by research, particularly for intensively managed pine plantations. Generally, we know how to avoid significant impacts, and nutrients are routinely added to raise productivity in plantation systems. In a nationwide study of long-term (10-year) soil productivity impacts of carbon removals conducted on 26 sites, even complete removal of all aboveground organic matter, including the forest floor, had no consistent effects, with the single exception of a reduction in the 10-year growth of aspen stands as compared to bole-only removal (*33*). Effects of removals on soil carbon sequestration are less clear. A review and meta-analysis of harvesting studies found that harvesting generally had little or no effect on soil carbon, although whole-tree harvesting caused a slight decrease (*34*). Fertilization and invasion by native N-fixers significantly increased soil carbon. It seems reasonable that more complete biomass removals could have a greater negative effect on the soil carbon pool, but this would likely vary by levels of removals, types of equipment used, as well as soil, site, and stand characteristics. Thus, further research is needed to identify the forest type and soil combinations where potential deficiencies may exist that could be triggered by biomass harvesting at certain levels of intensity and frequency of removal.

Deadwood serves important ecological functions in forest ecosystems (*35*). The amount of deadwood is affected by harvesting. Deadwood serves as habitat for a variety of organisms, including fungi, mosses, liverworts, vertebrates, and invertebrates, as well as regenerating plants. Deadwood alters site water balance and water quality, both through storage and release of water and by reducing runoff and erosion. Deadwood may also support biological nitrogen fixation, and it contains nutrients that are cycled back into the soil. Because of the important roles played by deadwood, some level of deadwood should be retained to protect these functions (*20*). The roles played by deadwood differ substantially among forest types and regeneration systems, making it unlikely that a universal standard for leaving deadwood on-site would serve all combinations. Nevertheless, recommendations seem to be settling on 30–35 percent retention (*20*) without regard for potential fire hazard.

There is also concern that biomass removals can reduce biological diversity. In planted forests this could result from more complete biomass removals or from greater ground disturbance. Retaining standing dead and downed stems can provide habitat for diverse ground-level flora and fauna (*36*, *37*). More complete removals in naturally regenerated forests could remove non-commercial yet ecologically valuable tree and shrub species; this concern can be addressed locally by appropriate retention guidelines. Also addressed in guidelines (see below) is the rule to avoid removals entirely from areas of high conservation value, such as old forests, wetlands, and rare habitats (*20*).

Benefits from Biomass Removals

Fire hazard can be reduced and the vigor of remaining trees can be increased by biomass removals from overstocked stands resulting from fire suppression (*22*), cessation of grazing, or abandoned management. The financial value of forests can be increased by providing markets for thinnings (*38*), especially by removing small-diameter and non-merchantable stems and by improving growth on otherwise suppressed stems, particularly in naturally regenerated eastern hardwoods. Removals can also aid in forest restoration efforts where there are goals to alter stand composition or structure. Biomass harvest can provide revenue that can offset restoration costs (*39*) but may be more costly than other methods (e.g., pile and burn treatments to reduce hazardous fuels). Revenue for biomass can also help fund restoration following wildfire, insect or disease outbreaks (*40*), or weather-related disturbances such as ice or windstorms (*41*).

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Social (Institutional) Support and Limitations on Forest Biomass Supply

Policies and Regulations Influencing Renewable Energy Production [Some text adapted with permission from the 2009 and 2010 ECE/FAO Forest Products Market Review (*61*, *62*).]

A key factor that will be a driver in markets for wood feedstock for energy is the definition of “biomass” in legislation, which determines what materials can obtain an incentive for energy use. As a result, depending on the legislation, wood from different kinds of stands and different forest ownerships will or will not qualify for incentives to produce wood-based liquid fuels, heat, or power. The definition varies between the 2007 Energy Independence and Security Act (EISA 2007) (*42*); the Food, Conservation, and Energy Act of 2008 (PL 110-234, Farm Bill) (*43*); and numerous pieces of draft legislation currently being debated.

EISA 2007, which promotes biofuels production and sets a target for the United States to produce 21 billion gallons of advanced biofuels by 2022, allows wood biomass feedstock only from non-federal land—with the exception of material adjacent to buildings or public places. Allowable wood from non-federal land includes previously established actively managed tree plantations and slash or pre-commercial thinnings.

The Farm Bill, which supports biomass supply for energy and investments in biomass energy production, allows use of wood from federal lands taken to reduce fire hazard or improve forest health and any wood from non-federal land available on a renewable basis.

The 2008 Farm Bill and 2007 EISA have different nationwide restrictions on biomass that can be used. An analysis by the USDA Forest Service (*44*) for the U.S. South, for example, indicates that the biomass definition in the 2008 Farm Bill would allow forest biomass to be supplied from 189.7 million acres of forest land. The 2007 EISA would allow biomass from planted forest area, or 44.4 million acres. The 2007 EISA excludes biomass from 17.3 million acres of federal land and 145 million acres of non-planted forest land. The sources allowed by a prospective federal renewable electricity standard have not yet been determined. Discussion is ongoing on how to reconcile these and other definitions of forest biomass that could qualify for various federal incentive programs.

State-level policies also promote or limit forest biomass use for bioenergy. Common policy instruments used by state governments that influence wood biomass use for energy include (1) rules and regulations including renewable portfolio standards, (2) financial incentives, and (3) programs supporting research, outreach, and education (*45*). In addition, states have policies to support sustainable use of wood biomass, including (1) definitions of biomass that can be used for energy to meet regulatory targets or qualify for subsidies, (2) establishment of mandatory or voluntary best forest management practices for supplying wood biomass, and (3) requirement for a professional forest management plan before biomass can be removed and used to meet regulatory targets or qualify for subsidy.

Financial incentives are the most common policy instrument and used by at least 40 states, most commonly to support feedstock demand or supply or to lower cost of capital investments. Almost all are designed to support a range of renewable energy sources including wood or agricultural biomass, wind energy, or solar energy and do not focus exclusively on wood. For example, Georgia exempts agricultural and wood biomass from the state's sales and use taxes (*46*).

Rules and regulations are the second most common type of instrument. Thirty-six states and the District of Columbia have Renewable Portfolio Standards (RPS) or Renewable Fuels Standards (RFS) (*47*) that set targets for the percentage of energy generated (or publicly purchased) in the state that must come from renewable sources by certain dates. These targets are most commonly for percentage of electric power (RPS) from renewables but sometimes represent the percentage of transportation fuels (RFS) from renewables. In most cases they are fixed percentages for given years. In some cases targets are flexible. In 2010, governors of 25 states have endorsed the vision of the 25 by '25 organization to provide 25 percent of electric power from renewables by 2025, although individual state legislation varies. For example, Missouri requires 2 percent for 2011–2013, 5 percent for 2014–2017, 10 percent for 2018–2020, and 15 percent for 2021 and thereafter (*48*).

Public service programs including education, research, and outreach are provided by 18 states, are least common, and are not specifically directed to support wood bioenergy. Support is given to develop a range of technologies and for programs to provide technical assistance to a range of businesses.

State policies supporting sustainability of wood biomass supply include biomass definitions that are intended in part to limit competition for wood inputs with the forest products industry and to support use of underutilized material. A minority of states have developed wood biomass harvesting best management practices, including Maine, Michigan, Minnesota, Missouri, and Wisconsin. For example, guidelines for Wisconsin are to retain tops and limbs (with <4-in. diameter) from 10 percent of trees in the harvest area and to not remove the forest litter layer, stumps, or roots (*49*).

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Regulations on Forest Practices

Amounts of biomass supply will be influenced by forest practice regulations that are specified by most state governments. These regulations currently focus on harvesting for conventional products and often derive their legal standing from federal water quality legislation (*50*, *51*), although some states have gone further in prescribing practices (*52*). According to Shepard (*28*), 12 states have regulatory procedures with mandatory Best Management Practices (BMPs) or require permits for timber harvest, and most others have voluntary BMPs and enforcement against polluters. The states with the most prescriptive regulations are California, Oregon, and Washington (*53*). With high rates of compliance to voluntary BMPs (*54*), this system is sufficient for conventional forest practices (*28*). States vary in their emphasis on what to protect in BMP guidelines, partially a reflection of diverse forest ecosystems, land ownership, and levels of timber harvesting. In the eastern United States, for example, northeastern states emphasize the influence of harvesting on nutrient depletion and stormflow; BMPs in mountainous states (Appalachians and Ouachita) focus more on stormflow, erosion, and sedimentation; in the low relief Lake States and Coastal Plain, maintaining site productivity is of greater concern (*51*).

The most common restrictions on harvesting within BMPs relate to water quality protection and focus on defining buffers around water bodies and limiting activity within the buffer zone. Most attention has been directed toward perennial streams and rivers, wetlands, and lakes with less attention to headwaters and ephemeral streams (*55*).

Several states have developed additional guidelines for biomass harvesting in existing forest stands; Maine, Minnesota, Missouri, Pennsylvania, and Wisconsin have published at least draft versions. The primary concern addressed in these guidelines is the potential effect of removing greater amounts of biomass than would be removed in conventional harvest, and the common remedy is to specify what and how much material should be left on-site. Because the state forest lands in Pennsylvania are certified by the Forest Stewardship Council (FSC), their guidelines reference the FSC Appalachia Regional Standard but with greater specificity.

Evans and Perschel (*56*) reviewed the various state biomass removal guidelines and concluded that they all addressed issues of dead wood, wildlife and biodiversity, and water quality and riparian zones. Generally, these guidelines called for retention of 15–30 percent of slash on-site, with various amounts of snags and cavity trees retained. All called for avoiding sensitive areas (e.g., shallow soils, unique habitats, areas of conservation value). Water quality and riparian zones were assumed to be protected by existing BMP guidance for conventional harvesting, although several states reiterated the guidance for biomass harvests. Where differences arose they related to local or regional concerns for silviculture (for example, regeneration was mentioned by Minnesota, Pennsylvania, and Wisconsin) and disturbances; several states proscribed biomass removals where dispersal of harmful insects or diseases was a concern. Maintaining soil fertility and site productivity was addressed specifically in the guidelines from Maine and Wisconsin; in the first case, reference is made to soil drainage classes, and in the second case, 17 specific nutrient-poor soils where removals are to be avoided. Maine, Minnesota, Pennsylvania, and Wisconsin guidelines all protect the forest floor and root systems from removal.

Thus, most biomass BMPs focus on restricting the amount of biomass that can be removed from the site. Three states (Minnesota, Missouri, Pennsylvania) place restrictions on re-entry to the site for biomass removal following conventional harvest, thereby favoring integrated operations. As biomass harvesting becomes more commonplace and more biomass-fueled power plants are proposed, it is likely that more states will adopt biomass harvesting guidelines, although more than likely, guidance will be along the lines already promulgated by these five eastern states. The effect of these BMPs on biomass supply is difficult to estimate because of the wide variability in stand conditions (biomass potentially available), harvesting methods, and differences among the state BMPs. We expect that restrictions on re-entry and retention requirements for snags and deadwood will have the most effect on biomass supply. The amount of slash removed will probably be more sensitive to costs of handling and transport than BMP restrictions. These assumptions should be tested to refine future estimates of biomass supply.

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In addition to the influence of state BMPs on biomass removals, there will be the influence of the major voluntary forest certification systems in limiting biomass removals. Certification under the Forest Stewardship Council (FSC) and the Sustainable Forestry Initiative (SFI) both require monitoring and avoidance of loss of soil nutrients among other factors that will limit biomass removals from forest land (*57*, *58*). In North America, SFI and FSC have certified 181 and 109 million acres, respectively (*59*). SFI also recognizes an additional 202 million acres certified by the Canadian Standards Association (CSA) and American Tree Farm System (ATFS). The idea of certifying well-managed private forests in the United States began with creation of the American Tree Farm System in 1941 based on a set of forest management principles and inspections each 5 years. In 1993, FSC was created to prevent deforestation globally, with an initial focus on tropical deforestation. SFI was created by the U.S.-based American Forest and Paper Association—an industry trade group—to respond to the FSC and address public concerns about sustainability (*60*). SFI has since become an independent non-profit organization with third-party auditing, as has FSC. Forest landowners benefit from certification by obtaining market access and market share, depending on the credibility of the system's environmental claims. Because these certification systems call for monitoring of soil effects of wood biomass removal of all types, they will inevitably play a role in limiting such removals as demand for wood biomass from forest land increases.

Summary

For biomass supply from forests to be sustainable, it needs to contribute to the broader sustainable development of forestry. Sustaining forestry includes maintenance of environmental services such as water, biodiversity, and carbon storage as well as timber products, forest-based community development,

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forest-based recreation, and forest non-timber products. Biomass supply can contribute, depending on forest area and conditions, in several ways. These include reducing fire hazard and insect and disease attack, aiding in restoration of forest composition and structure, enhancing forest growth, providing revenue to support treatments, providing revenue to support communities, and offsetting net greenhouse gas emissions. But there are biological, economic, and social limitations on amounts of supply. Biological limitations on amounts and kinds of biomass that can be removed vary by forest type and condition, by ownership (public, private), and by how stands are regenerated (planted, natural). Biological limitations are needed to maintain water supply, soil nutrients, and biodiversity. There are economic limitations that depend on the forest type, condition, and biomass to be removed, and removals may be more or less costly or could exceed expected revenues. Improvement and adaptation of harvesting technology can help address both biological and economic limitations. Estimates of U.S. total forest-based biomass supply given current understanding of biological and economic limitations suggest supply of about 45 million dry tons per year or more at $44/dry ton at forest roadside or mill gate. This estimate assumes there are no restrictions on forest sources of biomass that can be used. With increased price, the supply increase would vary by source. The increase from logging residue and thinnings would be limited by harvest for sawlogs and pulpwood as well as harvest costs, whereas the increase from pulpwood would be limited by owner stumpage price, harvest costs, and demand of pulpwood for pulp and panels. Social (institutional) targets and limitations in the form of federal and state legislation will support or limit wood biomass supply. These actions include a federal renewable cellulosic fuel target that includes biomass source restrictions, state-level renewable energy portfolio standards that may support biomass use for electric power production, and state-level forest practice guidelines that influence the kinds and amounts of biomass that can be removed from particular forest types and conditions. Understanding of biological and economic limitations and benefits is still developing, particularly at regional and local levels. Social targets and limitations could also change. A factor that could help accelerate development of understanding or change in social targets and limitations would be substantial increase in prices for fossil fuels that could be replaced by wood bioenergy or biofuels.

References

1. World Commission on Environment and Development (WCED). *Our Common Future*; Oxford University Press: Oxford, UK, 1987; p 400.
2. USDA Forest Service. *National Report on Sustainable Forests—2003*; FS-766; USDA Forest Service: Washington, DC, 2004; p 139.
3. Perlack, R. D.; et al. *Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-Ton Annual Supply*; USDOE, Oak Ridge National Laboratory: Oak Ridge, TN, 2005; p 58.
4. Smith, W. B.; et al. *Forest Resources of the United States, 2007*; GTR-WO-78; USDA Forest Service: Washington, DC, 2009; p 336.

Downloaded by 89.163.35.42 on June 8, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch001

5. Howard, J. *U.S. Timber Production, Trade, Consumption and Price Statistics, 1965 to 2005*; FPL-RP-637; USDA Forest Service: Madison, WI, 2007; p 91.
6. Howard, J. L.; McKeever, D. B. *U.S. Forest Products Annual Market Review and Prospects, 2006–2010*; FPL-RN-0315; USDA Forest Service: Madison, WI, 2010; p 11.
7. USDOE. *Annual Energy Outlook 2010*; Report #DOE/EIA-0383(2010); USDOE: Washington, DC, 2010.
8. BRDI. *Increasing feedstock Production for Biofuels: Economic Drivers, Environmental Implications, And the Role of Research*; Biomass Research and Development Initiative: Washington, DC, 2008, p 148.
9. Haynes, R. W., et al. *The 2005 RPA Timber Assessment update*; Gen. Tech. Rep. PNW-GTR-699; USDA Forest Service: Portland, OR, 2007, p 212.
10. Abt, R. C.; Galik, C. S.; Henderson, J. D. *The Tear-Term Market and Greenhouse Gas Implications of Forest Biomass Utilization in the Southeastern United States*; CCPP 10-01; Nicholas School of the Environment, Climate Change Policy Partnership, Duke University: Durham, NC, 2010, p 32.
11. Western Governors Association. *Strategic Assessment of Bioenergy Development in the West—Spatial Analysis and Supply Curve Development*; Western Governors Association: Denver, CO, 2008, p 86.
12. Richardson, J., *Bioenergy from Sustainable Forestry: Guiding Principles and Practice*; Kluwer Academic Publishers: Boston, MA, 2002; p 344.
13. Hanegraaf, M. C.; Biewinga, E. E.; VanDer Bijl, G. *Biomass Bioenergy* **1998**, *15* (4–5), 345–355.
14. Reijnders, L. *Energy Policy* **2006**, *34*, 863–876.
15. Volk, T. A.; et al. *Front. Ecol. Environ.* **2004**, *2*, 411–418.
16. Groom, M. J.; Gray, E. M.; Townsend, P. A. *Conserv. Biol.* **2008**, *22*, 602–609.
17. Keoleian, G.; Volk, T. *Crit. Rev. Plant Sci.* **2005**, *24*, 385–406.
18. Powers, R. F. *New For.* **1999**, *17* (1), 263–306.
19. Manley, A. *Biomass Bioenergy* **1995**, *9*, 89–105.
20. Janowiak, M. K.; Webster, C. R. *J. For.* **2010**, *108* (1), 16–23.
21. Markewitz, D. *For. Ecol. Manage.* **2006**, *236* (2–3), 153–161.
22. Neary, D.; Zieroth, E. *Biomass Bioenergy* **2007**, *31*, 638–645.
23. Conrad, J. L., IV; et al. *For. Pol. Econ.* **2010**, *12* (6), 399–406.
24. Butler, B.; Shao, M.; Kettredge, D. B.; Catanzaro, P. *North. J. Appl. For.* **2010**, *27* (4), 151–159.
25. Rummer, B.; Prestemon, J.; May, D.; Miles, P.; Vissage, J.; McRoberts, R.; Liknes, G.; Shepperd, W. D.; Ferguson, D.; Elliot, W.; Miller, S.; Reutebuch, S.; Barbour, J.; Fried, J.; Stokes, B.; Bilek, E.; Skog, K. *A strategic assessment of forest biomass and fuel reduction treatments in western states;* s.l.; USDA Forest Service, Research and Development: Washington, D.C., 2003; p 18.
26. Farnsworth, A.; et al. *Biomass Bioenergy* **2003**, *24* (4–5), 269–276.
27. Lindenmayer, D. B.; et al. *Science* **2004**, *303*, 1303.
28. Shepard, J. P. *Biomass Bioenergy* **2006**, *30* (4), 378–384.

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29. Webb, E., *A Preliminary Assessment of the State of Harvest and Collection Technology for Forest Residues*; ORNL/TM-2007/195; USDOE Oak Ridge National Laboratory: Oak Ridge, TN, 2008.
30. Marshall, D. J., *Synthesis of Knowledge of Hazardous Fuels Management in Loblolly Pine Forests;* Gen. Tech. Rep. SRS-110; USDA Forest Service, Southern Research Station: Asheville, NC, 2008, p 43.
31. Baker, S. A.; Westbrook, M. D., Jr.; Greene, W. D. *Biomass Bioenergy* **2010**, *34* (5), 720–727.
32. Nordfjell, T.; et al. *Scand. J. For. Res.* **2010**, *25* (4), 382–389.
33. Powers, R. F.; et al. *For. Ecol. Manage.* **2005**, *220*, 31–50.
34. Johnson, D. W.; Curtis, P. S. *For. Ecol. Manage.* **2001**, *140* (2–3), 227–238.
35. Harmon, M. E.; Franklin, J. F.; Swanson, F. J.; Sollins, P.; Lattin, J. D.; Anderson, N. H.; Gregory, S. V.; Cline, S. P.; Aumen, N. G.; Sedell, J. R.; Kienkaemper, G. W.; Cromack, K., Jr.; Cummins, K. W. *Adv. Ecol. Res.* **1986**, *15*, 133–302.
36. Brockerhoff, E.; et al. *Biodiversity Conserv.* **2008**, *17* (5), 925–951.
37. Carnus, J. M.; et al. *J. For.* **2006**, *104* (2), 65–77.
38. Polagaye, B. L.; Hodgson, K. T.; Malte, P. C. *Biomass Bioenergy* **2007**, *31* (2–3), 105–125.
39. Stanturf, J. A. In *Restoration of Boreal and Temperate Forests*; Stanturf, J. A.; Madsen, P., Eds.; CRC Press: Boca Raton, FL, 2005; pp 3–11.
40. Fettig, C. J.; et al. *For. Ecol. Manage.* **2007**, *238* (1–3), 24–53.
41. Stanturf, J. A.; Goodrick, S. L.; Outcalt, K. W. *For. Ecol. Manage.* **2007**, *250* (1–2), 119–135.
42. U.S. Congress. *U.S. Energy Independence and Security Act of 2007*; Public Law 110-140, U.S. Government Printing Office: Washington, DC, 2007. http://www.gpo.gov/fdsys/pkg/PLAW-110publ140/pdf/PLAW-110publ140.pdf.
43. U.S. Congress. *Food, Conservation, and Energy Act of 2008*; Public Law 110-234, U.S. Government Printing Office: Washington, DC, 2008. http://www.gpo.gov/fdsys/pkg/PLAW-110publ234/pdf/PLAW-110publ234.pdf.
44. USDA Forest Service. *The 2007 Energy Act versus the 2008 Farm Bill—a Comparison of Policy Definitions*, 2009. http://srsfia2.fs.fed.us/All%20States%20and%20Regions.pdf (cited September 9, 2010).
45. Aguilar, F. S.; Saunders, A. *J. For.* **2010**, *108* (3), 132–140.
46. Georgia, State of. *To amend Code Section 48-8-3 of the Official Code of Georgia Annotated, relating to exemptions from sales and use tax, so as to provide for an exemption regarding the sale or use of biomass material ... 2006*. Georgia House Bill 1018; Georgia, 2006.
47. North Carolina Solar Center. *DSIRE—Database of State Incentives for Renewables and Efficiency*, 2010. http://www.dsireusa.org/ (cited December 16, 2010).
48. Missouri, State of. *Renewable Energy Standard*; Missouri Revised Statutes Chapter 393. Gas, Electric, Water and Sewer Companies, 2009. www.moga.mo.gov/statutes/chapters/chap393.htm.
49. Herrick, S. K., *Wisconsin's Forest Land Woody Biomass Harvesting Guidelines*; PUB-FR-435-2009, Wisconsin Department of Natural

Resources, Division of Forestry and Wisconsin Council on Forestry: Madison, WI, 2009.

50. Kilgore, M. A.; Blinn, C. R. *Water, Air, Soil Pollut.: Focus.* **2004**, *4* (1), 203–216.
51. Aust, W. M.; Blinn, C. R. *Water, Air, Soil Pollut.: Focus.* **2004**, *4* (1), 5–36.
52. Ellefson, P. V.; Kilgore, M. A.; Granskog, J. E. *For. Pol. Econ.* **2007**, *9* (6), 620–632.
53. Ice, G.; et al. *Water, Air, Soil Pollut.: Focus.* **2004**, *4* (1), 143–169.
54. Ice, G. *J. Environ. Eng.* **2004**, *130* (6), 684–689.
55. Blinn, C. R.; Kilgore, M. A. *Water, Air. Soil Pollut.: Focus.* **2004**, *4* (1), 187–201.
56. Evans, A. M.; Perschel, R. T. *For. Guild.* **2009**.
57. Forest Stewardship Council. *FSC-US Forest Management Standard*; (v1.0), July 8, 2010. http://www.fscus.org/images/documents/standards/FSC-US%20Forest%20Management%20Standard%20v1.0%20with%20FF%20 indicators.pdf (cited December 20, 2010).
58. Sustainable Forestry Initiative. *Requirements for the SFI 2010-2014 Program—Tidance*, January 2010. http://www.sfiprogram.org/files/pdf/sfi_requirements_2010-2014.pdf (cited December 20, 2010).
59. Sustainable Forestry Initiative. *SFI and FSC Certification in North America—a Summary Comparison*, January 2010. http://www.sfiprogram.org/files/pdf/SFI_FSC_comparison_2010.pdf (cited December 20, 2010).
60. Hansen, E.; Fletcher, R.; Cashore, B.; McDermott, C. *Forest Certification in North America*; EC 1518, Oregon State University Extension Service: Corvallis, OR, 2006, p 11.
61. UNECE/FAO Timber Section. *Forest Products Market Review*; ECE/TIM/SP/24; United Nations: Geneva, Switzerland, 2009, p 165.
62. UNECE/FAO Timber Section. *Forest Products Annual Market Review*; ECE/TIM/SP/25; United Nations: Geneva, Switzerland, 2010, p 165.

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Chapter 2

Woody Biomass from Short Rotation Energy Crops

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Short rotation woody crops (SRWCs) are ideal for woody biomass production and management systems because they are renewable energy feedstocks for biofuels, bioenergy, and bioproducts that can be strategically placed in the landscape to conserve soil and water, recycle nutrients, and sequester carbon. This chapter is a synthesis of the regional implications of producing four genera of short rotation energy crops as feedstocks for fuels, chemicals, and fibers set in the rich history of research and development of these purpose-grown trees in the United States. The four genera include: *Populus* (cottonwoods, poplars, aspens), *Salix* (willows), *Pinus* (southern pines), and *Eucalyptus* (eucalypts). Key aspects of the production systems are discussed, including tree biology, genetics and tree improvement, and silvicultural management. The availability

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ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

of short rotation woody biomass is evaluated on the basis of maintaining sustainability at multiple scales. Current efforts to maximize production are described. Overall, sustainable production of fuels, chemicals, and fibers from woody biomass depends on a combination of feedstocks from both forests and plantations; the importance of dedicated SRWC feedstock production systems is highlighted.

Keywords: cottonwood; ecosystem services; energy security; eucalypts; hybrid aspen; intensive forestry; poplar; southern pine; sustainability; willow; woody feedstocks

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General Introduction

Forest biomass constitutes ~30% of the total biomass that can be produced in the United States (U.S.), making adequate woody feedstock availability necessary for environmental and economic sustainability. Woody feedstock production is vital for achieving our National goal of 16 billion gallons of cellulosic ethanol by 2022 (*1*). Improved woody biomass production and management systems are needed to: maintain healthy forests and ecosystems, create high paying manufacturing jobs, and meet local/regional energy demands. Short rotation woody crops (SRWCs) are ideal for such systems because they are renewable energy feedstocks for biofuels, bioenergy, and bioproducts that can be strategically placed in the landscape to conserve soil and water, recycle nutrients, and sequester carbon (*2*). Also, these crops are ideal for genetic improvement because of their ease of propagation, relatively short generation time, and broad range of genetic variation. Such variability can, however, contribute to sub-optimal productivity if genotypes are not properly matched to conditions at specific sites of deployment (*3*). Sustainable production of fuels, chemicals, and fibers from woody biomass depends largely on understanding factors regulating genotype × environment interactions, in addition to components such as nutrient management, retention of biodiversity in the landscape, and proper weed and pest control.

The selection of species and the genetic improvement for use as a feedstock will have to take different approaches to serve the two biofuel platforms: 1) biochemical (sugar) and 2) thermochemical (pyrolysis). For the biochemical platform of fuel production trees have been seen by some as a less desirable feedstock because of their high lignin content and recalcitrance to digestion. However, trees with naturally low levels of lignin and high cellulose have been found, e.g. in the hybrid aspen clone 'Crandon' (*4*) and loblolly pine (*5*). Transgenic aspens have been produced with lignin levels reduced by over 50% (*6*). Furthermore, work conducted at the U.S. Forest Service, Forest Products Laboratory has found that native aspens (*Populus tremuloides* Michx. and/or *P. grandidentata* Michx., bulked feedstock) have very low recalcitrance and are particularly easy for enzymatic conversion to glucose (*7*). Recent studies have also shown that clone NE-222 (*P. deltoides* Bartr. ex Marsh × *P. nigra* L.) is much more conducive to enzymatic conversion relative to clone NM6 (*P. nigra*

× *P. suaveolens* Fischer subsp. *maximowiczii* A. Henry) (*8*). Moreover, wood biomass is the preferred feedstock for the pyrolytic production of bio-oils because high lignin, with its greater energy density, is a desired characteristic (*9*, *10*). "Lignin has less oxygen than carbohydrate (so there is less to remove) and higher energy density, meaning more energy content per ton of biomass processed" (*11*). Whether for fuels, chemicals, or fibers from woody biomass, it is necessary to understand the biology, genetics and tree improvement, and silvicultural management of candidate trees. Such factors are described below for four SRWC genera: *Populus* (cottonwoods, poplars, aspens), *Salix* (willows), *Pinus* (southern pines), and *Eucalyptus* (eucalypts) (Figure 1; Tables Ia−Ic.

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Short Rotation Energy Crops

Populus

Introduction

It has been projected that *Populus* SRWCs could be grown as an energy crop on at least 24 million ha of U.S. land that currently supports marginal or environmentally-risky agriculture (*12*), which is nearly 450 times that currently deployed in North America (Table II). More SRWC research and practice has been conducted with the genus *Populus* than any other taxa. In fact, the genome of the Pacific Northwest cottonwood (*P. trichocarpa* Torr. & Gray) was the first of any woody species to be sequenced (*13*). Many excellent compendiums of *Populus* work are available (*14–16*). However, most of that research and essentially all of the practice has focused on the paper, and more recently, solid wood industries. It is clear that bioenergy could be a third product derived from the larger trees now being grown in *Populus* SRWC stands. *Populus* SRWCs are quite ready to fit into a multi-output biorefinery system (*17*, *18*). However, if *Populus* is to be optimally grown solely for conversion to bioenergy, it is likely that rotations shorter than five years, redesigned plantation structure, and the use of coppice regeneration will be necessary. Commercial coppice systems for *Populus* are only in the early stages of development. Obtaining woody biomass from these new systems is the primary subject of this review.

The eastern cottonwood (*P. deltoides*) has been the backbone of biomass production research in the North Central and Mississippi River Valley regions (Table Ia). *Populus deltoides* is found in nature from North Dakota to Texas to North Carolina, particularly along streams (*19*), but also in places where disturbance has greatly reduced other plant competitors and there is ample moisture for germination and early growth. In the northern U.S., *P. deltoides* has been the female parent for most of the development of hybrid planting stock (*20*, *21*). The European (*P. nigra*), Asian (*P. suaveolens* subsp. *maximowiczii*), or Pacific Northwest (*P. trichocarpa*) cottonwood have been used as the male parent to achieve faster early growth and better rooting. However, in the eastern U.S. from Madison, WI southward most of these interspecific hybrids are too susceptible to Septoria canker (*Septoria musiva* Peck) to be grown on rotations much over five years in length (*22*, *23*). Clones with pure *P. deltoides* backgrounds

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are much safer to use if they can be readily established from cuttings. *Populus deltoides* clones selected for good field rooting of dormant cuttings have been developed for use in the southern U.S. (*24*), but rooting of such genotypes has been erratic at northern latitudes (*25*). In other cases, rooted cuttings can be produced in standard tree nursery beds and then transplanted as rooted stock to field sites; the extra cost is more than offset under some environment conditions by higher survival and establishment growth (*26*, *27*).

Extensive native stands of quaking aspen (*P. tremuloides*) are only found in the northern part of the Lake States and the Rocky Mountains (*28*), while bigtooth aspen (*P. grandidentata*) is found in the Lake States and as scattered clumps from Iowa to North Carolina (*29*). Early settlers brought the European white poplar (*P. alba* L.) with them as a yard tree and it is now naturalized and/or hybridized with bigtooth aspens in additional small groves scattered across the same area.

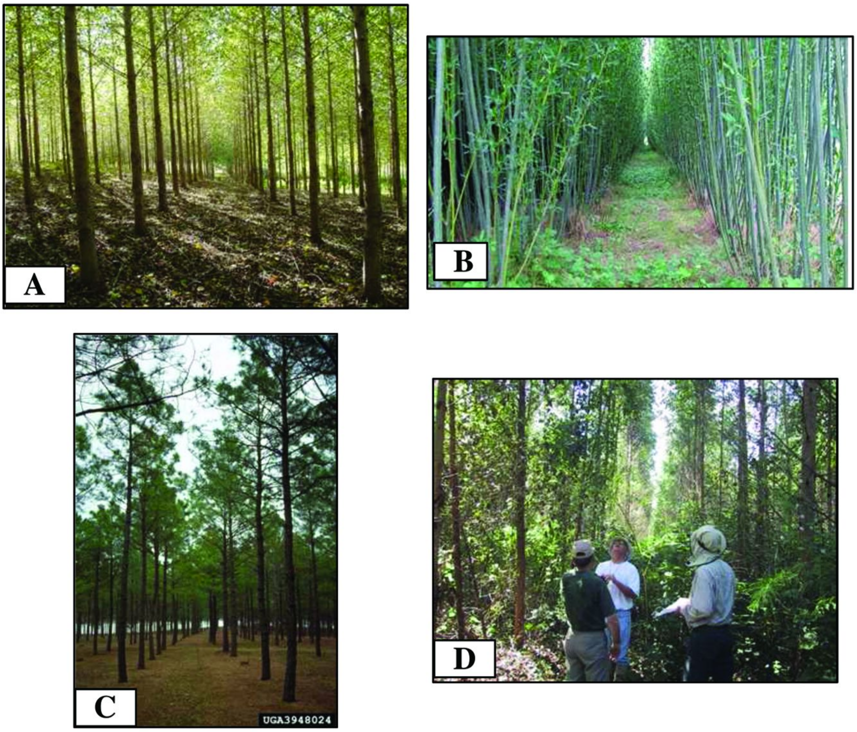

Figure 1. Plantations of Populus (A), Salix (B), Pinus (C), and Eucalyptus (D). Midrotation hybrid poplars in Minnesota (A; photo by Ron Zalesny, U.S. Forest Service); shrub willows in Upstate New York (B; photo by Tim Volk, State University of New York); loblolly pine after first thinning in Georgia (C; photo by University of Georgia –Bugwood); eucalypts on phosphate mined lands in Florida (D; photo by Don Rockwood, University of Florida). (see color insert)

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Table Ia. Species of potential short rotation energy crops that are most commonly used in different regions of the United States

	--------------- ***Populus*** ---------------						
Species	***Northeast***	***Lake States***	***Pacific Northwest***	***East Central***	***Midwest***	***Southeast***	***Southwest***
P. deltoides	Some	Some	In hybrids		Primary	Primary on alluvial sites	Experimental species and hybrids
P. nigra	In hybrids	In hybrids	In hybrids		Some in hybrids		Experimental as hybrids
P. suaveolens subsp. maximowiczii		In hybrids	Some in hybrids		Some in hybrids		
P. trichocarpa			Some and in hybrids				
P. alba		In Michigan	Some	Some in hybrids	Primary	Some in hybrids	Experimental as hybrids
P. grandidentata		Some in hybrids	Some in hybrids	Some in hybrids	Some in hybrids	Some in hybrids	Experimental as hybrids
P. tremula		Some in hybrids	Some in hybrids		Some in hybrids		

Table Ib. Species of potential short rotation energy crops that are most commonly used in different regions of the United States

-------------- Salix --------------	
Species / Parentage	***Clone*** *(currently recommended for SRWCs in the northeastern United States)*
S. purpurea	Allegany, Fish Creek, Onondaga
S. sachalinensis × *S. miyabeana*	Canastota, Sherburne
S. purpurea × *S. miyabeana*	Millbrook, Oneida
S. viminalis × *miyabeana*	Otisco, Owasco, Tully Champion
Salix × *dasyclados*	SV1
S. sachalinensis	SX61
S. miyabeana	SX64
S. miyabeana	SX67

-------------- Pinus --------------

Special note: There are numerous families of *P. taeda* that are used according to site, latitude and longitude ranging from Virginia to eastern Oklahoma and Texas. Hybrids are not used.

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Table Ic. Species of potential short rotation energy crops that are most commonly used in different regions of the United States

	---------------Eucalyptus ---------------	
Species	***Peninsular Florida / Hawaii***	***Lower Southeast***
E. grandis	Yes	No
E. amplifolia	No	Yes
E. benthamii	No	Yes
E. macathurii	No	Yes
Hybrids	Yes	No

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Table II. Description of industrial *Populus* plantations being grown in North America [adapted from Eaton (*76*)]

Region	*Land (ha)*	*Rotation (yrs)*	*Productivity (Mg ha^{-1} yr^{-1})*	*Primary use*
North Central	10,125	10 to 12	6.7 to 13.5	Pulp
Pacific Northwest	17,025	7 to 15	9.0 to 15.7	Solid Wood
Mississippi River Valley	11,350	8 to 10	4.5 to 6.7	Pulp
Canada	15,000	12 to 18	2.2 to 9.0	Pulp

Besides their potential use for biofuels, bioproducts, and bioenergy, the *Populus* species and hybrids have been extensively used for paper and particle board production with some work in making construction lumber (*30–32*). *Populus* genomic groups are also used in phytotechnologies to remove contaminants from soils and water (*33–35*). Utilizing wastewaters such as landfill leachate as irrigation and fertilization of *Populus* SRWCs may also contribute to the sustainability of growing the trees for energy-related applications (*36*, *37*).

Key aspects of *Populus* SRWCs are summarized here, including tree biology, genetics and tree improvement, and silviculture. Current efforts to maximize bioenergy production of cottonwoods, poplars, and aspens are described, and the opportunities afforded by *Populus* SRWCs are highlighted.

Biology

In North America, cottonwoods, poplars, and aspens occupy large distributional ranges with abundant genetic variation (*38*). Such genetic diversity is a hallmark of *Populus*, with variation present at the genus, sectional, species, and clonal level (*39–41*). There are 29 recognized species of *Populus* worldwide (*40*, *42*), with twelve species native to North America. Species of *Populus* are outcrossers with dioecious trees bearing either male or female pendant catkins (*38*, *40*, *43*). The ratio of male to female trees is generally 1:1, but variations exist at low altitudes with pistillate dominance and high altitudes with staminate dominance (*38*, *44*, *45*). The fast growing, deciduous, single-trunked trees are most notably researched and utilized for intensive management due to ease of rooting and vegetative propagation, quick establishment and fast growth leading to elevated rates of photosynthesis and transpiration, and the ability for some species to resprout (e.g., coppice) following harvesting or other top-killing events. Consequently, these three features make *Populus* genotypes particularly suitable for biomasss production, as well.

First, ease of rooting and vegetative propagation are key traits of the cottonwoods, particularly *P. nigra*, *P. trichocarpa*, and *P. suaveolens* subsp. *maximowiczii*. Tree breeders, silviculturists, and horticulturists take advantage of the propensity for these species to form adventitious roots by using unrooted hardwood stem cuttings as a common and inexpensive propagule in managed

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systems (*25*). Short stem pieces, about 30 cm long from one-year-old dormant material, quickly form shoots from dormant buds and roots from root primordia distributed throughout the stem (*27*, *42*, *46*, *47*). Additionally, adventitious roots form from callus, a wound-induced parenchymous growth at the base of the cutting. Riparian cottonwoods (i.e., *P. deltoides*) naturally reproduce asexually by branch breakage and crown damage. Branch sprouting and adventitious root formation facilitate tree survival (*43*). While some genotypes root well from unrooted cuttings, one of the chief drawbacks with *P. deltoides* is that rooting from unrooted, dormant hardwood cuttings is often erratic. In addition, a type of planting stock that is commonly used for *P. deltoides* is rooted cuttings with multiple lateral roots and some residual stems (usually not greater than one meter in height), or rooted cuttings reared in a greenhouse or growth chamber (see above).

Second, the leaves have a flattened, or otherwise flexible, petiole that gives them their characteristic quaking or fluttering appreance with the slightest of breezes. It has been postulated and to some extent proven that this helps the trees maximize photosynthetic rates across layers of leaves that would otherwise be shaded. Whatever the mechanisms, the *Populus* trees are usually the fastest growing components of temperate deciduous forests (Figure 1).

Third, in the cottonwoods, the ability to resprout is confined primarily to the stumps and it diminishes with age and frequent coppicing (*27*, *48*). In one of the few studies of repeated coppicing, cottonwoods planted on 0.6 × 1.2-m or 1.2 × 1.2-m spacings and cut on two-year cycles had high sprout vigor after the first cut, diminishing vigor after each of the next two cuts, and substantial mortality and loss of vigor beyond that (*48*). In most aspens, all or most of the sprouting comes from the roots, with root suckering following disturbance being much more important than seedling establishment. The sprouting potential is present in one-year-old seedlings, but tends to increase with age until the trees decline in vigor (*28*, *29*, *49*). The European white poplar sprouts well from the stump at young age as well as producing good root sprouts (*50*).

Genetics and Tree Improvement

The genus *Populus* is divided into six sections, based on specific ecological and morphological traits: *Abaso*, *Turanga*, *Leucoides*, *Aigeiros*, *Tacamahaca*, and *Populus* (*40*). The most important species for short rotation culture are in the sections *Aigeiros*, *Tacamahaca*, and *Populus*. Major barriers to hybridization occur among sections, with the success of artificial hybridization ranging from complete compatibility to complete incompatibility (*41*). Intersectional hybrids of economic significance occur between *Aigeiros* and *Tacamahaca* (*51–54*), with greater success being obtained when species of *Aigeiros* are used as females with *Tacamahaca* males (*51*, *52*, *55*). In addition, hybrids are common within and among species of the sections *Populus*; however, hybrids between this section and others are difficult to obtain (*56*, *57*).

The genus *Populus* was one of the first to undergo genetic improvement to meet human use goals (*14*). Poplar breeding was started in the U.S. in 1934

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(*20*). In the 1990s there were active breeding and/or selection programs for the *Aigeiros* and *Tacamahaca* sections in at least 21 states and 4 Canadian provinces. Unfortunately, only two active breeding programs remain in the U.S. As interest in biomass production grows, some programs may be restarted and new ones begun, but valuable time and often important germplasm has been lost. A summary of traits important to SRWCs of bioenergy has been developed (i.e., ideotype) (*16*). In particular, emphasis will need to be placed on ease of rooting of dormant cuttings and the retention of stump sprouting vigor over several rotations. It is possible that *P. nigra*, *P. suaveolens* subsp. *maximowiczii*, and *P. trichocarpa* can contribute to sprouting ability, just as they have to improved rooting. In current uses of SRWC *Populus*, it is assumed that enough genetic improvement progress is made over the course of a rotation to warrant replacement with new clones after each harvest. New guidelines will likely be needed for shorter rotations focused on bioenergy.

Selection for biomass yield has usually focused on approximate stem volume using some form of a D^2H formula, often times with an initial selection based on height and then for diameter. That priority order was reversed in the improvement program at Iowa State University based on the hypothesis that the shade avoidance response of pioneer species like those within the genus *Populus* naturally selects for height growth at the expense of growth allocated to diameter (*58*, *59*). A significant amount of the improvement in corn (*Zea mays* L.) yield was achieved by breeding out the shade avoidance (i.e., interplant competition) response (*60*). The concept still needs rigorous scientific testing, but the empirical results were good (*22*). However, there was one drawback, a small but significant negative correlation was found between diameter growth and specific gravity. It is theorized that selecting for faster growth tended to select for more vessel area to support higher levels of photosynthesis and rapid supply of soil nutrients to developing tissues. Selecting for optimal allocation of biomass will be even more important with frequent coppicing. One of the biggest benefits in this area will be increased understanding of the biology of wood formation and the ability to either down- or up-regulate the production of constituents like lignin to fit the needs of a particular conversion technology (*61*).

The cottonwoods are subject to some serious pest problems. For about the first three years of plantation development the cottonwood leaf beetle (CLB) (*Chrysomela scripta* Fabricius) can cause over 50% growth losses and deform stem growth (*62*). No significant genetic resistance has been found in *P. deltoides* clones and most hybrids. Fortunately, the insect can be controlled with careful crop scouting and spraying with an appropriate Bt formulation as each generation begins to emerge (*63*). Since bioenergy rotations may be entirely or mostly within the three-year period of high susceptibility, a renewed focus on developing transgenic insect resistance in *Populus* may be necessary (*64*).

A major leaf disease, poplar leaf rust (*Melampsora medusa* Thümen), can reduce cottonwood growth by 40%. Breeding and selection for resistance is possible and a single dominant resistance allele has been identified (*65*). As indicated earlier, the most serious disease of cottonwood-type hybrids in the eastern U.S. is Septoria canker. Hybrids with *P. trichocarpa* succumb to the disease after just a few years. *Populus nigra* and *P. suaveolens* subsp. *maximowiczii* hybrids may survive long enough for use in bioenergy rotations of

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five years or less (*66*). Pure *P. deltoides* clones are usually resistant to Septoria canker disease.

Moreover, an extensive program on aspen genetics and improvement was started by the Institute of Pulp and Paper Chemistry in Appleton, WI and later transferred as the Aspen and Larch Cooperative to Grand Rapids, MN. Significant progress was made on stem biomass production with the native aspens. In particular, the selection in the wild and the production through breeding of triploid aspens gave simultaneous and substantial increases in growth rate, fiber length and specific gravity (*67*). Polypoidy can also be induced through colchicine treatment and tissue culture techniques (*68*). A return to polyploidy research would likely give rapid and significant results in the improvement of biomass production. A program of interspecific hybridization with *P. alba* was initiated by the Appleton group with promising early results. However, a new disease called bronze leaf [*Apioplagiostoma populi* (E.K. Cash & Waterman) M.E. Barr] began to be seen in the hybrid clones (*69*, *70*) and the hybrid work was curtailed in Wisconsin and Minnesota; some improvement work with the native aspens continues.

Nature started its own interspecific breeding program in places where *P. alba* had been introduced into the vicinity of the bigtooth aspen. In the 1940s and 1950s several distinctive hybrid clones were discovered in southeastern Iowa (*71*). The most notable genotype is the ‘Crandon’ clone that has shown superior growth rates over a very wide geographic area from southern Minnesota to northern Alabama and east to West Virginia ((*3*, *72*); unpublished industry plantation results). Bronze leaf disease has not yet become a problem in Iowa and areas to the south and east. With the success of the Crandon hybrid aspen and the apparent absence of most insect and disease problems a modest breeding program was first conducted in 1990 to 1991 by Patrick McGovern, a private breeder in Michigan using a variety of *P. alba, P. grandidentata, P. tremula,* and F_1 hybrid parents. Progeny tests were established in Iowa and other nearby states under the U.S. Department of Energy’s Biofuels Development Program. Clonal selections were made from one of those trials and a clone test was established in 1996 (*73*) and harvested in 2008. Three of the top five clones in biomass production were pure *P. alba* selections, one was a *P. alba* × *P. tremula* hybrid and the other was a *P. alba* × *P. grandidentata* hybrid. Eleven of the new clones outperformed the Crandon clone. Specific gravity ranged from 0.28 to 0.43 g cm^{-3}. The two best stump sprouters were sibling clones from the *P. alba* × *P. grandidentata* cross. One *P. alba* family was produced with a heterozygous fastigiate male parent (Bolleana cv.). The best biomass producer of the family was a fastigiate clone that ranked seventh overall. However, the other fastigiate clones were poor biomass producers, as were the two normally branched clones (*74*).

Silviculture

Standard practices in traditional *Populus* SRWCs are well detailed in previous reviews (*27*) and are similar for energy plantations (especially for site preparation and vegetation management). Essentially all commercial cottonwood type SRWC plantations are established with unrooted cuttings from 15 cm (north) to 45 cm

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(south) long. To start aspen plantations, rooted plants can be produced in the nursery from segments 10 cm long and about 0.5 to 2 cm thick (*75*). A new method of rooting 10 cm long by $\leq$ 0.5 cm diameter dormant stem cuttings is under development.

Most cottonwood and aspen plantations have been planted on something close to a 3 × 3 m spacing with intensive weed control for one to three years, and harvest at 7 to 15 years (18 years in Canada) (Table II) (*76*). Yields for cottonwood and hybrid poplar stands range from 2.2 to 15.7 Mg ha^{-1} yr^{-1} on these rotations (Table II) (*3*, *27*). In contrast, a series of Crandon hybrid aspen yield trials in Iowa resulted in an average of 25 Mg ha^{-1} yr^{-1} on a 10-year rotation (*72*). However, to really maximize SRWC yields for bioenergy, much more work needs to be done in optimizing spacing and harvest age. For example, a Nelder spacing design was used with the Crandon hybrid aspen clone to determine the age:spacing:yield relationships for the first rotation (*77*), and a similar approach was used to study cottonwood yields as a function of spacing and number of coppice rotations (*48*). A limited amount of work has been done with *Populus* in testing the double row system of planting cuttings that has been used so successfully with willows (see below) (*78–80*) and more is needed on this and other innovative planting designs.

Extensive research has been done on managing root sprouts in native aspen stands (*49*). Unfortunately, research on SRWC root sprouts has only just begun (*81*). First year re-sprouting can result in over 200,000 stems ha^{-1} from a combination of stump and root sprouts with natural thinning starting during the first year. When the original plantations are harvested, a combination of both stump sprouts and root sprouts is produced. Observations of such mixed sprout origins suggest that stump sprouts have the fastest growth over the first one to two years, leading to significant mortality in adjacent root sprouts. After about two years, the stump sprouts begin to decline in health, vertical stability, and survival relative to the remaining root sprouts. Our results to date indicate that the yield per unit area harvested in a thinning at the end of the first year could be as much as 5.5 Mg ha^{-1} (root sprouts 3.3 Mg ha^{-1}, stump sprouts 2.2 Mg ha^{-1}) with no new establishment costs.

A new agroforestry study was initiated in 2009 by planting Crandon trees at a 3.0 × 3.6 m spacing into a matrix of forage triticale (*Triticale hexaploide* Lart.) established the previous fall. The triticale is harvested as a biomass crop in June leaving stubble for soil protection and making more soil water available to the trees. The triticale left unharvested next to the trees dies by early July, but remains standing for most of the rest of the season. Triticale biomass cropping between the tree rows can be repeated over the first few years until the trees dominate the site and go on to complete an 8- to 10-yr initial rotation. Alternatively, aspen root spread is being monitored so we can determine if a first-rotation stand can be converted to coppice production cycles after a only few years of harvesting triticale as the main biofuels feedstock. Lateral root spread of up to 2.4 m distal from stems was documented in the first year (*82*).

Not enough attention has been given to clonal deployment strategies with the genus *Populus* (*83*). The recommended deployment of willow clones from different diversity groups across the landscape is discussed below. The standard approach to *Populus* plantations has been to plant a mixture of monoclonal

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blocks, with each block being many hectares in size. Essentially all cottonwood type clones are planted this way, often wihout attention to diversity among adjacent clones. Deployment strategies for aspen plantations should also be further evaluated. The aspens may be more tolerant of interclonal competition and within block mixtures may provide a way to reduce the costs of both time and money during clonal testing (*84*). A mix of proven and promising clones can be established in commercial plantations. The best clones for each particular site will crowd out the less suited clones. When the first rotation stands are harvested, the majority of the sprouting should be from the most vigorous clones under the local environmental conditions and these naturally selected clones should continue to dominate in successive coppice rotations.

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Salix

Introduction

Shrub willow (*Salix* spp) has been developed as a perennial energy crop for the production of biomass in North America and Europe during the past 40 years (*37*, *85–87*). Willow research in North America started in southeastern Canada at the University of Toronto (*88*) and in Upstate New York in the mid 1980s (*86*). Currently, yield trials have been carried out or are under way in 15 states in the U.S. and in six provinces in Canada (Figure 1). Commercial nurseries have been developed to supply willow planting stock and over 400 ha of commercial scale plantings have been established in the U.S.

In addition to yield studies of different willow clones across sites, research in North America focused on various components of the production cycle, including nutrient amendments and cycling, alternative tillage practices, incorporating cover crops into these systems, density studies, harvesting systems development, and assessing pest impacts. A range of environmental characteristics of willow biomass crops have been assessed as well, including use of willow plantations by birds, changes in soil micro arthropod communities under willow, changes in soil carbon, and life cycle assessments of the system. The economics of the production system have been assessed and a cash flow model to reflect current production methods has been recently developed (*89*). In addition, breeding and selection programs for shrub willows have been developed in Canada and the U.S. The Canadian program was terminated in the early 1990s and the U.S. program that started in 1995 is still producing improved clones of willow for both the biomass production and agroforestry markets (*90*).

Key aspects of *Salix* SRWCs are summarized here, including tree biology, genetics and tree improvement, and silviculture. Current efforts to maximize production of willows are described, and the opportunities afforded by *Salix* SRWCs are highlighted.

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Biology

Species from the genus *Salix* are perennial, deciduous, shade intolerant pioneer species that are typically found primarily on moist soils along water ways in natural settings. Although willows inherently have a competitive advantage over other plants in wet conditions, they grow well on uplands and well-drained sites as long as competing vegetation is controlled during establishment and there is adequate rainfall (*91*). Willows are dioecious plants that produce catkins during the spring, typically before the leaves come out. The seeds contain silky white hairs, which allow them to be dispersed by wind, but they are typically only viable for a few weeks and require moist conditions to germinate and develop (*92*). Most willows are native to the temperate, boreal, and tundra regions of the northern hemisphere (*93*), with shrub willows being used for bioenergy crops coming primarily from temperate regions. Shrub willows have the ability to be propagated vegetatively and regrow after coppicing, which facilitates rapid multiplication and allows repeated harvests from a single planting.

Although weed competition and drought are the greatest threats to the establishment of a willow plantation, diseases and pests have the ability to impact the productivity of a crop. The fungus *Melampsora epitea* Thüm, which causes rust disease, has reduced yields in the United Kingdom and is being monitored in the U.S. It attacks leaves, causing them to senesce and drop prematurely. Rust is a concern for *S. eriocephala* Michx., but has limited impact on species and hybrids being used for biomass production in North America right now. Diseases that have less impact include anthracnose tip blight on *S. eriocephala* caused by *Colletotrichum* spp. and willow scab caused by *Physalospora miyabeana* Fukushi (*94*). Pests and insects documented in North America include Chrysomelid beetles, which include *Popollia japonica* Newman (Japanese beetle) and *Plagiodera versicolora* Laicharting (imported willow leaf beetle) in the U.S. (*95*). These beetles feed on the leaves of the willow and in the U.K. have been shown to decrease yields of susceptible willow clones (*96*, *97*). Stem-sucking insects such as *Tuberolachnus salignus* Gmelin (giant willow aphid) and *Pterocomma salicis* L. (black willow aphid) have been documented in shrub willows as well (*98*). To date none of these pests have had a measureable impact on yields of willow biomass crops in North America, but with increasing acreage, pest and disease pressures could create serious problems (*94*).

Genetics and Tree Improvement

Large genetic diversity and limited domestication of willow to date provide great opportunities to improve yield and other characteristics. The genus *Salix* comprises between 330 and 500 species (*93*, *99*, *100*) growing as trees, shrubs and dwarf shrubs. Polyploidy is common and some species are known to hybridize within the genus *Salix*. The species used for woody crop systems are primarily from the subgenus *Caprisalix* (Vetrix), which has over 125 species (*92*). These species share many characteristics, but differ in their resistance to pests and diseases and their architecture. Breeding and selection can improve yields across

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a wide range of site conditions, identify clones that are tolerant to diseases and pests, and identify growth forms that are more suitable for harvesting systems used for SRWC. Once superior willow clones are identified they can be multiplied rapidly using vegetative propagation.

Willow breeding started in Sweden and the U.K. in the 1980s and early 1990s, but many of the European clones did not perform well in North America due to damage caused by the potato leaf hopper (*Empoasca fabae* Harris). Willow breeding in North America started at the University of Toronto (UofT) in the 1980s and focused on heritability and genetic variation of native species such as *S. eriocephala*, *S. exigua* Nuttall., *S. lucida* Mühl., *S. amygdaloides* Anders., *S. bebbiana* Sarg., *S. pellita* Anders., *S. petiolaris* Smith, and *S. discolor* Mühl. Early studies with *S. eriocephala* showed that limited gains would be possible when breeding and selecting for height, diameter and yield (*101*, *102*). Breeding started in 1998 at SUNY-ESF with a variety of species including *S. eriocephala*, *S. sachalinensis* F. Schmidt, *S. purpurea* L., or *S. dasyclados* Wimm. with *S. miyabeana* Seemen and intraspecific crosses of *S. purpurea*. In four plant plot selection trials the highest yielding improved clone produced 77% more biomass than the reference clone 'SV1' (*S. dasyclados*) in the second rotation (*94*). First rotation yields of improved clones in small plot yield trials have been greater than reference clones and have ranged from 10.2 to 13.6 Mg ha^{-1} yr^{-1}. Previous studies indicate that there are different strategies for obtaining high biomass production among groups of willow species and clones (Table III) (*103*). These results indicate that there is a large potential to make use of the wide genetic diversity of shrub willows to improve yields with traditional breeding and selection.

Improving yields will make willow a more economically attractive crop for marginal lands. Increasing yields by 17% (from 12 to 14 Mg ha^{-1} yr^{-1}) improves the internal rate of return (IRR) for willow biomass crops by 51% (from 5.5 to 8.3%) (*89*). First-year rotations of willow have produced yields of 8.4 to 11.6 Mg ha^{-1} yr^{-1} (*86*, *104*, *105*) and second-year rotations yields are about 35% higher on average (*106*).

Silviculture

Willow can be grown on marginal agricultural lands in temperate regions. It should be planted in fully prepared open land, where weeds have been controlled. Typically field preparation starts the fall of the year before planting and includes a combination of mechanical and chemical techniques. Planting takes place in the spring between the end of April and the beginning of June. Erosion during establishment year has been successfully reduced through the use of cover crops, such as *Secale cereale* L. (winter rye) (*107*). Studies to evaluate conventional tillage, no tillage and conservation tillage methods are under way as well.

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Table III. Differences in morphological traits of *Salix* clones with high biomass production in short rotation woody crop systems [adapted from Tharakan et al. (*103*)]

Species group (variety name)	*Mean stem diameter (cm)*	*Number of stems per stool*	*Wood specific gravity (g cm^{-3})*	*Leaf area index*	*Specific leaf area (cm^2 g^{-1})*	*Foliar N (g kg^{-1})*
S. purpurea, *S. dasyclados* (SV1)	1.2	10.7	0.43	3.9	125.6	20.7
S. sachalinensis (SX61), *S. miyabeana* (SX64, SX67)	1.6	5.9	0.39	4.5	129.7	14.7

Willows are planted as unrooted dormant hardwood cuttings using tractor drawn planters. The early planters were adapted from potato planters to plant 20 to 25 cm cuttings. Currently available planters from Sweden (Step Planter) and Denmark (Egedal Energy Planter) use 2 to 3 m whips and cuts them into 15 to 20 cm long sections and inserts them into the ground. Shrub willows are typically planted in a double row system at 15,000 plants ha^{-1}, with 1.5 m between the double rows, 0.76 m within the double-row and 0.61 m between the plants within the rows, to allow clearance for harvesting and cultivation machinery. Trials are underway to examine the potential of reducing planting density of new clones to decrease establishment costs while maintaining yields. Single (*108*) and triple row systems have also been used in some plantings. After the first year the willows are cut at about 5 cm above the soil after leaf drop. This process is called coppicing and increases the number of stems from 1 to 3 up to 8 to 13 depending on the clone (*103*). Coppicing facilitates future harvests, increases yields and helps to control weed competition due to earlier canopy closure.

Typically willows are harvested every 3 to 4 years using forage harvesters with a specially designed cutting head (*109*). Whole stem harvesters and modified bailers have also been developed. Following harvest the plants will re-sprout the following spring when they are typically fertilized with about 100 kg nitrogen (N) ha^{-1} of commercial fertilizer or organic sources like manure or biosolids (*109*). Projections indicate that the crop can be maintained for seven rotations before the rows of willow stools begin to expand to the point that they are no longer accessible with harvesting equipment.

Pinus

Introduction

Southern pine species are an important component of the forest resources of the U.S. South, which is one of the most important timber producing regions globally (*110*). Of the over 74 million ha total timberland in 11 southern states (excludes Oklahoma and Kentucky), 27.5 million ha are classified as softwood types and another 8.5 million ha are oak-pine types (*111*). Pine plantations account for 15 million ha, more than half of the area of softwood forest. The intensification of pine plantation silviculture is one of the remarkable stories of U.S. forestry (*112*) and it sets the stage for discussing the potential role that short-rotation pine could play in woody bioenergy development.

The historical development of intensive pine forestry has been described as a process of crop domestication (*112*, *113*). Several authors have described this process from multiple perspectives (*112–116*). In broad outline, intensification has involved tree improvement, seedling quality and stocking control, site preparation, management of competing vegetation, fertilization, and pest management (*114*, *116–118*). Research on soil-site and growth and yield have ensured that the potential gain in productivity from these basic improvements is realized by properly deploying improved planting stock and appropriate silvicultural interventions (*112*). More than 95 percent of the seedlings planted in the South are genetically improved loblolly and slash pines (*Pinus taeda* L. and *P. elliottii* Engl., respectively) (*118*).

The distinguishing feature of timberland in the South is that private ownership predominates. Fully 87% of all timberlands in the South and 95% of the pine plantations were privately owned in 2007 (*111*). These numbers obscure structural shifts in ownership that have already occurred and shifts in timberland location projected to occur in the near term (*119*). The nearly 20% of timberland formerly held by vertically integrated forest products companies (industrial land) is now largely owned by real estate investment trusts (REIT), timber investment and management organizations (TIMO), pension funds, and other financial institutions (*120*, *121*). Although non-industrial private landowners own a small percentage of the pine plantations, they account for a substantial area. The demographics of these owners suggest that this land area will soon see further ownership fragmentation through sales and generational transfers (*120*, *122*). Not all plantations will be managed at the same intensity so differing landowner objectives will affect whether biomass production for bioenergy is feasible and if so, which silvicultural system is adopted.

Rising population and increasing per capita wealth in the South will likely drive changes in land use (*119*). This combination of factors suggests that the center of intensive pine silviculture will shift westward as urbanization proceeds on the coastal plain and in the Piedmont (*121*). Indeed, Zhang and Polyakov (*111*) suggest that by 2027, plantation area will decline in Florida, Georgia, North and South Carolina by 3.5% and increase in Alabama, Mississippi, Arkansas, and Louisiana by 30%. While federal and state policy and market forces will determine where and how much land will be available for developing pine bioenergy plantations, these projected shifts into relatively drier areas will affect how accurately we can predict potential yield from currently available genetic material and silvicultural prescriptions. Possibilities for bioenergy production are several (*12*), including continued use of harvesting residues from traditional operations (see Skog et al. of this volume; (*123*)), possibly including more complete removal from sites; integrating bioenergy with production of other

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products; and developing dedicated bioenergy plantations on marginal agricultural land or cutover forestland. In all cases, current intensive management systems will continue to be developed.

A recent report from the U.S. Departments of Agriculture and Energy provides the best national estimate of how much biomass could be available for energy production from cropland and forests (*12*). They projected that approximately 342 million dry tons of biomass annually could come from converting from 16 to 24 million ha of agricultural land to perennial crops. This would add an additional 2.4 to 5.8 quadrillion BTUs of renewable energy (as compared to the 2.9 quads that came from biomass in 2003, which accounted for almost 3% of total energy consumption in the U.S. in that year).

Key aspects of *Pinus* SRWCs are summarized here, including tree biology, genetics and tree improvement, and silviculture. Current efforts to maximize production of southern pines are described, and the opportunities afforded by *Pinus* SRWCs are highlighted.

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Biology

Of the four most common southern pine species, shortleaf pine (*P. echinata* Mill.) is the most widespread but it is not planted and little has been done to develop improved material, which probably disqualifies it from consideration for SRWCs. Slash pine (*P. elliottii*) was the backbone of the naval stores industry but its susceptibility to rust (*Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. fusiforme (Cumm.) Burds. & Snow) generally has limited planting to Florida and south Georgia. Since this is the area projected to lose plantation area due to land use change (*111*, *121*), slash pine also is an unlikely candidate for widespread SRWC planting. Longleaf pine (*P. palustris* Mill.) was once the most widespread of the southern pines and restoration of longleaf is a popular topic. Nevertheless, longleaf exhibits slow early growth; although the establishment problems once experienced with longleaf have largely been overcome, the growth habit of initially allocating most growth to belowground biomass limits its utility for short rotation plantings.

The rapid early growth and responsiveness to amendments of loblolly pine (*P. taeda*) has made it the pine of choice for intensive silviculture in the South (*124–127*) (Figure 1; Table Ib, and Table IV). The response to fertilization may depend on whether significant competition from shrubs or hardwoods is controlled (*116*, *126*), and interactions among site and genetics have been shown (*128*, *129*). Because there is much installed capacity and knowledge of loblolly pine intensive silviculture (*112*, *113*, *116*, *129*), it is the most likely prospect for bioenergy development. One drawback relative to hardwood species, however, is that loblolly pine does not coppice.

Fast-growing, densely planted SRWC pine plantations will be challenged by endemic organisms, including Nantucket pine tip moth (*Rhyacionia frustrana* Comstock), fusiform rust, and southern pine beetle (*Dendroctonus frontalis* Zimmerman). Tip moth affects rapidly growing material (*130–132*) and can result in sustained growth loss (*133*, *134*). Control of competing vegetation and

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use of insecticides that reduce levels of natural enemies may result in damage from insects that otherwise do not reach economically damaging levels (*131*). Fusiform rust has co-evolved with southern pines and will continue to challenge tree breeders to develop rust resistant planting stock. Mortality from fusiform rust is highest on young trees, and treatments to increase growth such as fertilization increases rust incidence. Thus, resistance to fusiform rust will be of prime concern in establishing SRWC pine plantations and recent advances in understanding the fusiform rust-loblolly pine pathosystem promise better strategies for avoiding losses (*135*). Southern pine beetle, another endemic disease, should present less of a problem in SRWC pine plantations as it more typically attacks larger, older trees. If there is an outbreak in the vicinity, however, even young pine stands can be decimated.

Genetics and Tree Improvement

Most of the loblolly pine seedlings planted in the U.S. are of genetically improved stock (*118*, *129*). As of 2002, 59% of all loblolly pine plantations were established as single, open-pollinated family blocks; on industry lands this was 80% (*129*). As material from advanced breeding programs becomes available for operational planting, most large organizations deploy this material to their best sites. In 2002, companies reported less than 1% of their material was planted as full-sib families (*118*). Individual clones from rooted cuttings or tissue culture have been planted in experimental plots and early results indicate that genotype × environment interactions are relatively unimportant under current conditions (*129*).

Biotechnology promises further advances in domesticating loblolly pine. Opportunities exist to enhance growth rates and reduce rotation (harvesting) ages, convey greater pest and disease resistance, and to produce trees with chemical and structural characteristics optimized for chemical processing (i.e., designer trees), as well as accelerating traditional breeding programs (*136*). Biotechnology may be used to genetically engineer these and other traits, but will require parallel efforts to overcome public misperceptions about the technology as transgenic trees become available for operational deployment.

Silviculture

Intensive pine silviculture in the South includes a variety of site preparation treatments appropriate to conditions, weed control, fertilization, and several thinnings during a typical 25-year rotation (Table IV). Before mergers and land divestitures in the 1990s (*112*, *120*), some forest industry companies concentrated on producing pulpwood-size material and experimented with shorter rotations that eschewed thinning and included tip moth control, obtaining peak annual growth increment of 3.1 to 3.6 Mg ha^{-1} yr^{-1} at ages 10 to 12 on some sites (*112*, *114*). Mean annual increment of 2.1 to 2.7 Mg ha^{-1} yr^{-1} was routinely obtainable on most sites (*127*, *137*).

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Table IV. Silvicultural prescriptions and potential yields from intensively-managed *Pinus* plantations in the southern United States

Age (yrs)	*Sawlog*[a]	*Pulpwood*[b]	*Comment*
0	Site preparation	Site preparation	Aerially applied chemical, followed by combination plow[c]
1	Plant	Plant	Improved 1-0 bareroot seedlings; Flexwood system plants two levels of improved seedlings
1	Herbaceous weed control	Herbaceous weed control	Product and rate depend on site
2	Fertilize	Fertilize	Diammonium phosphate (DAP), 225 kg ha^{-1}
1 to 3	Tip moth control	Tip moth control	As needed[d]
5	Fertilize	----------	135 kg N ha^{-1}; 17 kg P ha^{-1},[e]
6	----------	Fertilize	135 kg N ha^{-1}; 17 kg P ha^{-1}
9	Fertilize	----------	135 kg N ha^{-1}; 17 kg P ha^{-1}
10	----------	Fertilize	135 kg N ha^{-1}; 17 kg P ha^{-1}
13	Thin and fertilize	----------	135 kg N ha^{-1}; 17 kg P ha^{-1}
14	----------	Harvest	138 to 152 Mg ha^{-1} (pulpwood[f])
17	Fertilize	----------	135 kg N ha^{-1}; 17 kg P ha^{-1}
19	Thin	----------	
21	Fertilize	----------	135 kg N ha^{-1}; 17 kg P ha^{-1}
25	Harvest	----------	134 to 157 Mg ha^{-1} (pulpwood/ energywood[g])

[a] Modeled silvicultural prescription from Allen et al. (*114*) [b] Modeled silvicultural prescription from Borders and Bailey (*127*); fertilizer rates are taken from Allen et al. (*114*) [c] Combination plow, usually pulled by a tractor with a V-blade to clear slash on cutover sites; plow combines coulter wheel and subsoiler followed by two bedding disks [d] Tip moth control is not generally operational but has been shown to provide an economically significant response in intensively-managed plantations (*112*). [e] On some loamy and sandy sites, studies have shown response to potassium and boron; some companies include complete micronutrient application where response is suspected. [f] Range is the average and optimistic level in Borders and Bailey (*127*). [g] Range is the medium and high level in Allen et al. (*114*); total yield was 228 Mg ha^{-1} with 157 Mg ha^{-1} pulpwood/energywood at the medium level and 309 Mg ha^{-1} total yield with 228 Mg ha^{-1} pulpwood/energywood at the high level.

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There is little published information on loblolly pine SRWC but the information from studies comparing species and genotypes in dense plantings is illustrative, with loblolly generally out-performing slash and with a strong interactive effect of density and genotype for loblolly pine. Burkes et al. (*125*) compared four planting densities (740, 2220, 3700, and 4400 trees ha^{-1}) and found no significant difference in stemwood production after 4 years between the two denser spacings. Stem biomass growth in the fourth growing season was 17.4 Mg ha^{-1}. Adegbidi et al. (*138*) reported biomass accumulation and partitioning in four intensively managed loblolly pine stands planted at 1495 trees ha^{-1} with the same improved family. Stemwood growth after four years was 10.1 Mg ha^{-1} yr^{-1}, which accounted for 34% of net primary production. Roth et al. (*128*) obtained total aboveground biomass after five years from intensive treatments of 55 Mg ha^{-1} with 2990 trees ha^{-1} as compared to 37 Mg ha^{-1} for operational management at the same planting density. Varying the planting density also had an effect; under the intensive treatment, average stem diameter was 13.1 vs. 10.9 cm for 1334 and 2990 trees ha^{-1}, respectively (*128*). Most significantly, they identified the need for nutrient amendments on poor sites earlier in the rotation than is typical of conventional intensive silviculture.

Modifications to integrate bioenergy into current intensive pine silviculture have been proposed, including dual-cropping and intercropping. In dual-cropping, the pine stand is established and managed to intentionally produce both biomass for energy and crop trees for roundwood products (*139*). Direct-seeding pine between the rows of a traditional pine plantation produced about 10.2 Mg ha^{-1} of biomass for energy after 5 years without adversely affecting the crop trees (*139*). Another version of dual-cropping called FlexStand involves planting two pine genotypes together, a very elite genotype for the crop tree and an improved genotype for biomass (*140*). The advantage is that the lower value biomass is also a lower cost seedling. In the intercropping system, an annual bioenergy crop such as switchgrass is planted between the rows of the pine crop trees (*141*). The full details of spacing, harvesting, and the economics of these systems are being studied.

Eucalyptus

Introduction

Bioenergy could be the highest contributor to global renewable energy in the short to medium term, with SRWC *Eucalyptus* playing a major role (*142*). *Eucalyptus* species can be widely planted to produce abundant biomass, but their planting may require various incentives. Several biomass conversion technologies are operational, and other biomass opportunities include biorefineries, carbon sequestration, and small, distributed energy systems. Brazilian experience suggests that *Eucalyptus* bioenergy can be produced efficiently and sustainably in the U.S. (*143*). Biomass-derived electricity and liquid fuels may compete with fossil fuels in the short-term, most likely by using integrated gasifier/gas turbines to convert biomass to electricity (*144*).

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
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Bioenergy currently constitutes ~2.8% of the U.S. energy production, with ~60% of this due to the forest products industry (*145*); by 2030, forest bioenergy could double. *Eucalyptus* SRWCs can provide renewable energy feedstocks for biofuels, bioenergy, and bioproducts for tropical and subtropical regions of the U.S., namely Florida and portions of other southeastern states, Hawaii, and California (Table Ic). At present, ~50,000 ha of *Eucalyptus* SRWCs are planted in California, Hawaii, and Florida.

Short rotation woody crop *Eucalyptus* could be grown for bioenergy on up to 100,000 ha in Hawaii following guidelines from a research and development program in the 1980s (*146*). *Eucalyptus saligna* Smith in 5- and 6-year rotations and an 8-year *Eucalyptus/Albizia* mix produced 20.2, 18.6, and 26.9 or more Mg ha^{-1} yr^{-1}, respectively. Chipped *Eucalyptus* biomass was most expensive for the 5-year rotation and least for the 6-year rotation. Short- and long-term improvement programs were not implemented before program termination in 1988, but subsequent efforts by various agencies have identified promising genotypes in several species. About 9,000 ha of *Eucalyptus* plantations established since 1996 are producing over 40 m^3 ha^{-1} yr^{-1} in the most productive areas (*147*).

Due to Florida's challenging climatic and edaphic conditions, much SRWCs emphasis has been on *Eucalyptus* tree improvement for adaptability to infertile soils and damaging freezes. *Eucalyptus grandis* Hill ex. Maiden is now grown commercially in southern Florida for mulchwood and can be used in central Florida (Figure 1) (*148*), while *E. amplifolia* Naudin is suitable from central Florida into the lower Southeast. On suitable sites and/or with intensive culture, they may reach harvestable size in as few as three years (*149*). *Eucalyptus* SRWCs are promising for cofiring in coal-based power plants in central Florida. By combining superior clones (*150*), suitable culture (*148*), innovative harvesting (*148*), and efficient conversion, these two species have considerable potential in Florida. As in other SRWC development situations, research on genetics, spacing, fertilization, planting, control of pests and diseases, forest management, etc., will be essential for achieving high SRWC productivity.

Key aspects of promising *Eucalyptus* species are summarized here, including biology, genetics and tree improvement, silviculture, and the opportunities they afford as SRWCs.

Biology

Eucalypts are successful SRWCs because of their fast growth and environmental tolerance due to attributes such as indeterminate growth, coppicing, lignotubers, drought/fire/insect resistance, and/or tolerance of soil acidity and low fertility, and many have desirable wood properties for bioenergy production. While the biological traits of all currently promising *Eucalyptus* SRWCs are somewhat common, *E. grandis*, *E. amplifolia*, *E. benthamii* Maiden et Cambage, *E. macarthurii* Deane et Maiden, and *Eucalyptus* hybrids still differ in significant ways (Table V)

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Table V. Relative biological characterizations of *Eucalyptus* species with high potential for bioenergy production in the southeastern United States. See text for species' authorities and additional descriptions

Characteristic	*E. grandis*	*E. amplifolia*	*E. benthamii*	*E. macarthurii*	*Hybrids*
Max. growth rate yr^{-1}	6 m	5 m	5 m	4 m	7 m
Site tolerance	Wide	Moderate	Wide	Moderate	Wide
Fertilizer response	High	Moderate	Moderate	Moderate	High
Age to seed production	Short	High	High	High	n/a
Quantity of seed produced	High	Limited	Moderate	Moderate	n/a
Ease of vegetative propagation	Easy	Difficult	Difficult	Difficult	Easy
Cold tolerance	Limited	High	High	High	Moderate
Coppicing	Limited	High	High	High	High
Wood density	Moderate	Moderate	Moderate	Moderate	Moderate

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The indeterminate growth of eucalypts coupled with a range in inherent cold tolerance puts many otherwise desirable species at risk in subtropical climates with highly variable temperatures and freezes, such as the southeastern U.S. Species/sources whose natural distributions include similar low temperatures, temperature fluctuations, and rainfall patterns tend to match exotic environments best.

As monoecious species with varying tendencies to inbreed, eucalypts often produce non-uniform seedling populations. Thus, seedling-based plantations can be less productive than clonal plantations due to poor growth of as much as 25% of the trees.

Eucalypts that vegetatively propagate easily are ideal for combining superior growth, pest resistance, cold tolerance, etc. Clonal plantations of highly selected *E. grandis* and related hybrids, for example, are common across the world.

Genetics and Tree Improvement

Eucalyptus species are ideal for genetic improvement because many typically propagate easily, have short generation intervals and broad genetic variability, and may be genetically engineered. The species/cultivars/hybrids with documented bioenergy potential in the Southeast are described below and in Table Ic and Table V. A total of 79 accessions of 35 species are being evaluated in 2010 and 2011 at 13 sites from Texas in the west to North Carolina in the north and south through Florida (*151*).

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E. grandis. Genetic improvement of *E. grandis* for Florida conditions started in the 1960s. Substantial improvements in the species' growth, form, and freeze resilience were achieved, culminating in the release in 2009 of the commercial cultivars E.nergy™ G1, G2, G3, and G4 (*150*). While G1, G2, G3, and G4 have exceptional growth rate, stem form, freeze tolerance, and coppicing ability compared to 4th-generation *E. grandis* seedlings, the four cultivars have important differences in these characteristics, their genetics, and wood properties. Planted at five locations throughout peninsular Florida in 2009, the cultivars survived well, were up to 6.1 m tall in 8 months, and typically tolerated the exceptionally cold weather of January and February 2010. Their deployment expanded in 2010, and they will be widely available as commercial SRWCs in southern, central, and even northern Florida. Research is developing even more superior *E. grandis* cultivars with desirable wood properties and pest resistance.

E. amplifolia. Genetic improvement of *E. amplifolia* for Florida began in the 1980s. Significant gains have been made in growth, form, and freeze resistance, and 1st- and 2nd-generation seedling seed orchards are established. Clonal selections have been made and are under evaluation. Improved trees were generally undamaged by the January and February 2010 freezes. In an intensive culture study near Sumterville, 7-year-old *E. amplifolia* yielded 42 green Mg ha^{-1} yr^{-1}.

E. benthamii. The species has a limited range in Australia, but trees within that range are abundant. The species is tolerant to freezing temperatures and is planted in southern Brazil on high elevation sites. *Eucalyptus benthamii* was first tested in the southern U.S. in the early 1990s by Westvaco Corp. As in Brazil, it shows very good tolerance to freezing temperatures and has survived temperatures as low as -12 °C with minimal damage (ArborGen internal data). Genetic improvement in the U.S. has been limited to open-pollinated mother tree tests and within-family tree selection to establish seedling seed orchards. The first progeny tests of this material were planted by ArborGen and collaborating companies in 2009. Seedlings from the top families have produced up to 34.8 green Mg ha^{-1} yr^{-1} at 6 years in a trial in South Carolina. Average yields are predicted to be 27 to 36 Mg ha^{-1} yr^{-1} on a 7 year rotation based on ArborGen and MeadWestvaco internal data. This species has demonstrated a wide site and climate adaptability in Coastal Plain plantings from Texas to South Carolina.

E. macarthurii. *Eucalyptus macarthurii* has been widely tested and planted in South Africa. It was also one of four species identified as having the most cold tolerance among several species tested by North Carolina State University at multiple locations in Florida, Georgia, South Carolina, and Alabama. Great improvements in forest productivity and frost tolerance have been attained since the first seedlots were planted in the U.S. Recently, new genetic trials of seedlings from improved mother trees are providing encouraging results. Significant improvements can be made in frost tolerance and growth rates by selecting the best families for plantation establishment. In a 0.8-ha planting in South Carolina, 8-year-old *E. macarthurii* produced 22 green Mg ha^{-1} yr^{-1} of clean chips.

Eucalyptus hybrids. A genetically engineered hybrid of *E. grandis* × *E. urophylla* S.T. Blake with genes for cold tolerance, lignin biosynthesis, and/or fertility is currently in the U.S. regulatory approval process (*152*). In field tests,

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these trees have survived temperatures as low as 6 °C, which allows them to be planted south of Interstate-10. The variety is well known for its high quality fiber and also excels at biomass production. Furthermore, this *Eucalyptus* can be planted on marginal lands. In traditional pulpwood management systems, this hybrid is predicted to produce 34 to 43 green Mg ha^{-1} yr^{-1} on a seven year rotation. Planted in a biomass management system, this productivity can potentially be increased to 43 to 52 green Mg ha^{-1} yr^{-1}. Further incorporation of growth genes may result in a four year rotation.

Efforts to identify regions of the *Eucalyptus* genome that regulate biomass growth and wood quality have been largely successful (*143*). As biotechnology and genomics research have allowed for once inconceivable achievements, genetic and genomics studies will likely discover most genes regulating significant portions of the heritable variation of biomass productivity and wood property traits. Ultra low sequencing reaction volumes suggest that a *Eucalyptus* genome could be sequenced in less than a day for a few hundred dollars, making it then possible to identify superior genotypes based on their genotype across multiple critical loci. For example, cinnamoyl CoA-reductase is a significant determinant of fiber properties in *Eucalyptus*. Several current studies are identifying genes of value for bioenergy, particularly those involved in the lignin and carbohydrate/cellulose pathways. Once genotyping assay methods are sufficiently cost effective to permit rapid screening of progenies in breeding programs, genotypes that combine the optimal alleles for bioenergy can be reliably identified.

Silviculture

Eucalyptus is promising for bioenergy production in the southern U.S. Cost estimates for production and delivery range from \$65 to \$79 dry Mg^{-1} (*153*). Since productivity greatly affects delivered cost, high productivity sites and systems should be favored. Shorter rotation lengths, more freeze-tolerant trees, and higher stand tree density combined with good silvicultural practices can improve productivity.

The silviculture of all *Eucalyptus* SRWCs involves many common necessities and considerations: site selection and preparation, propagule quality, weed control, spacing, fertilization, rotation length/harvest time, and coppicing. Failure in any one of these areas will severely impact productivity. For example, poor site selection, inadequate site preparation, inferior propagules, lack of weed control, improper spacing, infertility, or wrong season of harvest can, at worst, each lead to failed plantations.

Site selection and preparation are the initial critical silvicultural choices. Naturally fertile sites are ideal for *Eucalyptus*, with former and marginal agricultural sites often being very suitable. Previously forested sites need to be thoroughly cleared of debris and stumps. Poorly drained sites may require bedding and/or subsoiling. Nutrient deficiencies, most notably phosphorus (P), should be corrected. All these activities should be completed well before planting begins.

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Once the appropriate species and/or genotype is chosen for the site, propagule quality needs to be ensured. The ideal containerized propagule should have a well developed, solid root ball with a firm, upright stem about 30 cm in length. Underdeveloped or overdeveloped propagules are both undesirable for achieving good survival and growth.

Control of competition within eucalypt plantations can be more difficult and expensive than in pine plantations because of the sensitivity of seedlings to the herbicides. Efforts are continuing at University of Florida and Louisiana State University to either develop or recognize chemicals that are effective on weeds and more tolerable to crop trees. In the meantime, attention to detail in applying herbicides to eucalypt plantations is critical.

Herbicides for herbaceous weed control applied over the top of planted seedlings should be applied very early in the growing season. Based on specific site characteristics, herbicides can be broadcast over an entire tract by hand, helicopter, or rubber-tired equipment. Banded applications of herbicides, at least two feet on each side of the seedlings, can be applied with rubber-tired equipment or by hand.

Spacing influences tree size, yield, and time to harvest. Conventional densities of 1500 trees ha^{-1} produce larger trees over longer rotations, while higher SRWC densities such as 3000 trees ha^{-1} maximize per ha productivities in shorter rotations.

Fertilization can be critical to achieving high productivity. *Eucalyptus* typically responds linearly to additions of N and P, which often limits growth on poorer sites. Soil amendment with wastewaters and composted waste materials can be successful. Many species are sensitive to micronutrient deficiencies, particularly boron (B) and copper (Cu).

Rotation length/harvest time can be driven by tree size requirements and harvesting equipment. Very short rotation SRWC systems may be dictated by cost effective multirow harvesters with maximum stem diameter requirements of 10 cm. Time of harvest is important with species, e.g., *E. grandis*, that have seasonal windows for successful coppicing.

Coppicing success can also be influenced by genotype and planting density. Species such as *E. amplifolia* and *E. benthamii* coppice reliably and vigorously. Multistem coppicing can be minimized by genetic selection and planting density; however, in some instances thinning of coppice sprouts may be necessary.

Sustainability

Introduction

Short rotation energy crops are one of the most sustainable sources of biomass, provided they are strategically placed on the landscape and managed with cultural practices that conserve soil and water, recycle nutrients, and maintain genetic diversity (*2*). These woody biomass sources also provide benefits such as carbon sequestration, wildlife habitat, and soil stabilization (*154–156*). Plantations also provide opportunities to reduce pressure on native forests (*157*, *158*). Overall, the sustainability of producing woody biomass from short rotation

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energy crops depends on a combination of integrated social issues (e.g., the land use debate) and biological uncertainties (e.g., genotype × environment interactions) associated with production potential of each group of species described above (*159*). Economic barriers further complicate sustainability, especially in the face of heightened consumption of non-renewable resources (*160*).

Specific sustainability criteria include protecting the resource base, maintaining biodiversity, achieving carbon and climate neutrality, and attaining a positive energy balance. Protecting the resource base requires attention to maintaining productivity and avoiding off-site impacts from pesticide or nutrient movement (*161*). In general, the conversion from cropland to forests is a net environmental gain (*162–164*). Even short-rotation tree and shrub crops result in less soil disturbance from plowing than annual crops. This will decrease soil erosion and increase soil organic matter, which should maintain or increase site productivity (*165*). Potential negative effects are very specific to management systems and the machinery used to harvest and gather material. The main environmental concerns are whether high levels of removals and more trafficking by machinery would reduce future productivity by removing too many nutrients, lowering levels of soil organic matter, compacting soil, and increasing soil erosion. For example, such concerns have been addressed by research on intensive pine silviculture and generally, we know how to avoid significant impacts. Current voluntary forestry Best Management Practices could be modified for energy plantations (*166*). A further step short of government regulation would be to require a form of third-party certification for producing biomass for energy from planted forests (*167*). The development of bioenergy from food crops has escalated concerns for conversions of native forests and engendered criticism for increasing food costs (*168*). Neither scenario applies to the short rotation energy crops described here, with the possible exception of oak-pine stands that resulted when harvested SRWC pine plantations in the South were not re-planted and understory oaks on the site were released (*12*, *111*, *169*). It is also likely that marginal farmland will be converted to SRWCs in preference over cutover forestland because of lower establishment costs and social resistance (e.g., in the upper Midwest) (*170*). The total effects on carbon balance and energy efficiency depend on not only the production and harvesting of biomass but also on the energy it produces, which depends on the efficiency of the conversion technology and products that result. Overall, these sustainability criteria are affected somewhat differently throughout respective regions of the U.S. where specific purpose-grown trees are produced (Tables Ia–Ic) (*2*), yet overarching concerns are relevant irrespective of region. Although *Salix* is illustrated as a case study below, most of the sustainability principles described are true for *Populus*, *Pinus*, and *Eucalyptus* SRWCs, as well. The major differences are centered around silvicultural prescriptions inherent to the genera.

Case Study: *Salix*

Willow biomass crops are being developed in the Northeast as sustainable systems that simultaneously produce a suite of ecological, environmental and

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social benefits in addition to a renewable feedstock for bioproducts and bioenergy (*171*, *172*). The perennial nature and extensive fine-root system of willow crops reduces soil erosion and non-point source pollution relative to annual crops, promotes stable nutrient cycling and enhances soil carbon storage in roots and the soil (*173–176*). Replacing fertilizers with biosolids or wastewater can significantly lower both the costs of growing shrub willows (*177*) and the carbon inputs into the system (*178*). Herbicide use is also significantly lower for willow crops compared to a typical corn-alfalfa rotation, because its use is confined to the first year or two during establishment (*109*). Willow crops provide rural development benefits by diversifying farm crops, creating an alternative source of income for landowners, and circulating energy dollars through the local economy (*109*).

The recommended planting scheme for willow biomass crops is designed to maintain both genetic and structural diversity across a field and the landscape. Blocks of four or more willow clones from different diversity groups should be planted in each field so that the structural and functional diversity of the system across the field is improved and any potential impact associated with pests and diseases in the future is reduced. At the landscape level willow biomass crops will be in different stages of growth each year because they are managed on three year coppice cycles that are staggered to provide a steady flow of biomass to the end users and will increase the structural diversity of the system (*171*). A study of bird diversity over several years indicated that willow biomass crops provide good foraging and nesting habitat for a diverse group of birds. Thirty-nine species of birds visited the plantations and 21 used them for nesting (*179*). The number of bird species was similar to those in early succession habitats and intact eastern deciduous forests and increased compared to open agricultural land.

Life cycle analysis (LCA) of willows indicated that it is a low-carbon fuel, because the amount of CO_2 taken up by the plants during photosynthesis is almost equal to the amount of CO_2 released during production, harvest, transportation and conversion of the biomass to bioenergy (*178*). The cycle is balanced, because only the aboveground biomass is harvested and as such the carbon that is sequestered within the roots stays in the ground. Overall greenhouse gas (GHG) emissions from willow used for electicity generation are 95% lower than coal (*180*). Willow biomass crops have a large, positive net energy ratio. Accounting for all the energy inputs into the production system, results in a net energy ratio of 1:55 (*178*). Replacing commercial N fertilizers with organic amendments, such as biosolids, the net energy ratio can increase and range from 73 to 80 (*178*). Transporting the woody biomass 40 km from the edge of the field to a coal plant where it is co-fired with coal to generate electricity results in a net energy ratio of 1:11. If a gasification conversion system is used, the net energy ratio is slightly higher (*181*).

Conclusions

Overall, intensively managed plantations representing a diverse set of short rotation species are necessary to help achieve U.S. policy that mandates the production of 16 billion gallons of cellulosic biofuels by 2022 (*1*). Paramount to the success of this achievement is testing and identification of woody biomass feedstocks that grow fast, accumulate substantial biomass, and break down to sugars easily with energy efficient technologies. The four species groups described above possess a multitude of such traits that make them attractive candidates as dedicated energy crops. Decades of breeding and selection are beginning to show significant yield and disease resistance improvements in all groups, which has the potential to result in multiple socioeconomic, environmental and ecological benefits concurrently.

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References

1. U.S. Energy Independence and Security Act of 2007 (Pub. L. 110-140).
2. Vance, E . D.; Maguire, D. A.; Zalesny, R. S., Jr. *J. For.* **2010**, *108*, 183–192.
3. Zalesny, R. S., Jr.; Hall, R. B.; Zalesny, J. A.; McMahon, B. G.; Berguson, W. E.; Stanosz, G. R. *BioEnergy Res.* **2009**, *2*, 106–122.
4. Dickson, R. E.; Larson, P. R.; Isebrands, J. G. In *Proceedings of the 9th Central States Tree Improvement Conference*; Ames, IA, 1974; pp 21−34.
5. MacKay, J. J.; O'Malley, D. M.; Presnell, T.; Booker, F. L.; Campbell, M. M.; Whetten, R. W.; Sederoff, R. R. *Proc. Nat. Acad. Sci. USA* **1997**, *94*, 8255–8260.
6. Laigeng, L.; Zhou, Y.; Cheng, X.; Sun, J.; Marita, J. M.; Ralph, J.; Chiang, V. L. *Proc. Nat. Acad. Sci. USA* **2003**, *100*, 4939–4944.
7. Zhu, J. Y.; Pan, X.; Zalesny, R. S., Jr. *Appl. Microbiol. Biotechnol.* **2010**, *87*, 847–857.
8. Zhu, J. Y. U.S. Forest Service, Forest Products Laboratory, 2010; personal communication.
9. Radlein, D.; Sta, G.; Piskoriz, J.; Scott, D. S. *J. Anal. Appl. Pyrolysis* **1987**, *12*, 51 –59.
10. Mohan, D.; Pittman, C. U., Jr.; Steele, P. H. *Energy Fuels* **2006**, *20*, 848–889.
11. Brown, R. C. Iowa State University, 2010; personal communication.
12. Perlack, R.; Wright, L.; Turhollow, A.; Graham, R.; Stokes, B.; Erbach, D. Billion Ton Report by U.S. Departments of Energy and Agriculture: Washington, DC, 2005; p 78.
13. Tuskan, G. A.; DiFazio, S.; Jansson, S.; Bohlmann, J.; Grigoriev, I.; Hellsten, U.; Putnam, N.; Ralph, S.; Rombauts, S.; Salamov, A.; Schein, J.; Sterck, L.; et al. *Science* **2006**, *313*, 1596–1604.
14. Dickmann, D. I.; Stuart, K. W. *The Culture of Poplars in Eastern North America*; Department of Forestry, Michigan State University: East Lansing, MI, 1983; 168 p.
15. Stettler, R. F.; Bradshaw, H. D., Jr.; Heilman, P. E.; Hinckley, T. M. *Biology of Populus and Its Implications for Management and Conservation*; NRC

Research Press, National Research Council of Canada: Ottawa, ON, 1996; 539 p.

16. Dickmann, D. I.; Isebrands, J. G.; Eckenwalder, J. G.; Richardson, J. *Poplar Culture in North America*; NRC Research Press, National Research Council of Canada: Ottawa, ON, 2001; 397 p.
17. Ragauskas, A. J.; Williams, C. K.; Davison, B. H.; Britovsek, G.; Cairney, J.; Eckert, C. A.; Frederick, J.; Hallett, J. P.; Leak, D.; Liotta, C. L.; Mielenz, J. R.; Murphy, R.; Templer, R.; Tschaplinski, T. *Science* **2006**, *311*, 484–489.
18. Kleinert, M.; Barth, T. *Energy Fuels* **2008**, *22*, 1371–1379.
19. van Haverbeke, D. E. In *Silvics of North America, 2. Hardwoods*; Burns, R. M.; Honkala, B. H., tech. coords.; Ag. Handbook 654; USDA Forest Service: Washington, DC, 1990.
20. Schreiner, E. J. USDA Forest Service Res. Pap. WO-RP-11; 1971; p 19
21. Riemenschneider, D. E.; Stanton, B. J.; Vallée, G.; Périnet, P. In *Poplar Culture in North America*; Dickmann, D. I., Isebrands, J. G., Eckenwalder, J. E., Richardson, J., Eds.; NRC Research Press, National Research Council of Canada: Ottawa, ON, 2001; pp 43−76.
22. Riemenschneider, D. E.; Berguson, W. E.; Dickmann, D. I.; Hall, R. B.; Isebrands, J. G.; Mohn, C. A.; Stanosz, G. R.; Tuskan, G. A. *For. Chron.* **2001**, *77*, 245–253.
23. Weiland, J. E.; Stanosz, G. R. *Plant Dis.* **2007**, *91*, 1524–1530.
24. Mohn, C. A.; Randall, W. K.; McKnight, J. S. USDA Forest Service Res. Pap. SO-RP-62; 1970, p 17.
25. Zalesny, R. S., Jr.; Hall, R. B.; Riemenschneider, D. E. *Can. J. For. Res.* **2005**, *35*, 918–929.
26. Sulaiman, A-R. R. M.S. Thesis, Iowa State University, Ames, IA, 1986.
27. Stanturf, J. A.; van Oosten, C.; Netzer, D. A.; Coleman, M. D.; Portwood, C. J. In *Poplar Culture in North America*; Dickmann, D. I., Isebrands, J. G., Eckenwalder, J. G.; Richardson, J., Eds.; NRC Research Press, National Research Council of Canada: Ottawa, ON, 2001; pp 153−206.
28. Perala, D. A. In *Silvics of North America, 2. Hardwoods*; Burns, R. M., Honkala, B. H., Tech. Coords.; Ag. Handbook 654; USDA Forest Service: Washington, DC, 1990.
29. Laidly, P. R. In *Silvics of North America, 2. Hardwoods*; Burns, R. M., Honkala, B. H., Tech. Coords.; Ag. Handbook 654; USDA Forest Service: Washington, DC, 1990.
30. Hall, R. B.; Hilton, G. D.; Maynard, C. A. *J. For.* **1982**, *80*, 291–294.
31. Semen, E.; Kuo, M.; Su, Y. C.; Hall, R. B.; Stokke, D. D. *Wood Fiber Sci.* **2001**, *33* (1), 140–147.
32. Balatinecz, J. J.; Kretschmann, D. E. In *Poplar Culture in North America*; Dickmann, D. I., Isebrands, J. G., Eckenwalder, J. G., Richardson, J., Eds.; NRC Research Press, National Research Council of Canada: Ottawa, ON, 2001; pp 277−291.
33. Zalesny, R. S., Jr.; Bauer, E. O.; Hall, R. B.; Zalesny, J. A.; Kunzman, J.; Rog, C. J. *Int. J. Phytoremed.* **2005**, *7*, 177–197.
34. Zalesny, J. A.; Zalesny, R. S., Jr.; Wiese, A. H.; Sexton, B.; Hall, R. B. *Environ. Pollut.* **2008**, *155*, 72–80.

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Downloaded by STANFORD UNIV GREEN LIBR on June 8, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch002

35. Zalesny, J. A.; Zalesny, R. S., Jr.; Wiese, A. H.; Sexton, B. T.; Hall, R. B. *J. Sustainable For.* **2008**, *27*, 303–327.
36. Zalesny, J. A.; Zalesny, R. S., Jr.; Coyle, D. R.; Hall, R. B. *For. Ecol. Manage.* **2007**, *248*, 143–152.
37. Mirck, J.; Isebrands, J. G.; Verwijst, T.; Ledin, S. *Biomass Bioenergy* **2005**, *28*, 219–228.
38. Farmer, R. E., Jr. In *Biology of Populus and Its Implications for Management and Conservation*; Stettler, R. F., Bradshaw, H. D. Jr., Heilman, P. E., Hinckley, T. M., Eds.; NRC Research Press, National Research Council of Canada: Ottawa, ON, 1996; pp 33−55.
39. Rajora, O. M.; Zsuffa, L. *Genome* **1990**, *33*, 44–49.
40. Eckenwalder, J. E. In *Biology of Populus and Its Implications for Management and Conservation*; Stettler, R. F., Bradshaw, H. D. Jr., Heilman, P. E., Hinckley, T. M., Eds.; NRC Research Press, National Research Council of Canada: Ottawa, ON, 1996; pp 7−32.
41. Stettler, R. F.; Zsuffa, L.; Wu, R. In *Biology of Populus and Its Implications for Management and Conservation*; Stettler, R. F., Bradshaw, H. D. Jr., Heilman, P. E., Hinckley, T. M., Eds.; NRC Research Press, National Research Council of Canada: Ottawa, ON, 1996; pp 87−112.
42. Dickmann, D. I. In *Poplar Culture in North America*; Dickmann, D. I., Isebrands, J. G., Eckenwalder, J. G., Richardson, J., Eds.; NRC Research Press, National Research Council of Canada: Ottawa, ON, 2001; pp 1−42.
43. Braatne, J. H.; Rood, S. B.; Heilman, P. E. In *Biology of Populus and Its Implications for Management and Conservation*; Stettler, R. F., Bradshaw, H. D. Jr., Heilman, P. E., Hinckley, T. M., Eds.; NRC Research Press, National Research Council of Canada: Ottawa, ON, 1996; pp 57−85.
44. Farmer, R. E., Jr. *Silvae Genet.* **1964**, *13*, 116–118.
45. Kaul, R. B.; Kaul, M. N. *Southwest Nat.* **1984**, *29*, 265–269.
46. Luxova, M.; Lux, A. *Biol. Plant.* **1981**, *23*, 285–290.
47. Luxova, M.; Lux, A. *Biol. Plant.* **1981**, *23*, 401–405.
48. Geyer, W. A. *Biomass Bioenergy* **2006**, *30*, 778–783.
49. Zasada, J. C.; David, A. J.; Gilmore, D. W.; Landhäusser, S. M. In *Poplar Culture in North America*; Dickmann, D. I., Isebrands, J. G., Eckenwalder, J. G., Richardson, J., Eds.; NRC Research Press, National Research Council of Canada: Ottawa, ON, 2001; pp 119−151.
50. Hall, R. B.; Sabatti, M.; Scarascia-Mugnozza, G.; Anselmi, N. In *Proceedings of the Northern Forest Genetics Association* 1994, Vol. 2, pp 22−31.
51. Zsuffa, L. In *Symposium on Interspecific and Interprovenance Hybridization in Forest Trees, Proceedings of the 14th Meeting of the Canadian Tree Improvement Association*; Fowler, D. P., Yeatman, C. W., Eds.; Fredericton, New Brunswick, Canada, 1975; pp 107−123.
52. Guries, R. P.; Stettler, R. F. *Silvae Genet.* **1976**, *25*, 37–43.
53. Gaget, M.; Saïd, C.; Dumas, C.; Knox, R. B. *J. Cell Sci.* **1984**, *72*, 173–184.
54. Villar, M.; Gaget, M.; Saïd, C.; Knox, R. B.; Dumas, C. *J. Cell Sci.* **1987**, *87*, 483–490.

55. Eckenwalder, J. E. In *Poplar Culture in North America*; Dickmann, D. I., Isebrands, J. G., Eckenwalder, J. G.; Richardson, J., Eds.; NRC Research Press, National Research Council of Canada: Ottawa, ON, 2001; pp 331−382.
56. Ronald, W. G. *Silvae Genet.* **1982**, *31*, 94–99.
57. Eckenwalder, J. E. *Can. J. Bot.* **1984**, *62*, 325–335.
58. Hall, R. B. *Tree Physiol.* **1994**, *14*, 899–909.
59. Bae, H. H.; Kang, H. D.; Hall, R. B. *Korean J. Plant Res.* **2004**, *7* (1), 78–87.
60. Duvick, D. N. In *XX Congresso Nacional de Milho e Sorgo*; Goiania, Goias, Brasil, July 25−29, 1994.
61. Chapple, C.; Ladisch, M.; Meilan, R. *Nat. Biotechnol.* **2007**, *25* (7), 746–748.
62. Coyle, D. R.; McMillin, J. D.; Hall, R. B.; Hart, E. R. *Agric. For. Entomol.* **2002**, *4*, 293–300.
63. Coyle, D. R.; McMillin, J. D.; Krause, S. C.; Hart, E. R. *J. Econ. Entomol.* **2000**, *93*, 713–720.
64. Han, K.-H.; Meilan, R.; Ma, C.; Strauss, S. H. *Plant Cell Rep.* **2000**, *19*, 315–320.
65. Tabor, G. M.; Kubisiak, T. L.; Klopfenstein, N. B.; Hall, R. B.; McNabb, H. S., Jr. *Phytopathology* **2000**, *90*, 1039–1042.
66. Coyle, D. R.; Hart, E. R.; McMillin, J. D.; Rule, L. C.; Hall, R. B. *For. Ecol. Manage.* **2008**, *255*, 3365–3372.
67. Einspahr, D. W.; Benson, M. K.; Peckham, J. R. *Silvae Genet.* **1963**, *12*, 51–58.
68. Son, S. H.; Moon, H. K.; Hall, R. B. *Plant Sci.* **1993**, *90* (1), 89–94.
69. Newcombe, G.; Ostry, M.; Hubbes, M.; Perinét, P.; Mottet, M-J. In *Poplar Culture in North America*; Dickmann, D. I., Isebrands, J. G., Eckenwalder, J. G., Richardson, J., Eds.; NRC Research Press, National Research Council of Canada: Ottawa, ON, 2001; pp 249−276.
70. Smith, J. A.; Blanchette, R. A.; Ostry, M. E.; Anderson, N. A. *Am. Phytopathol. Soc.* **2002**, *86* (5), 462–469.
71. Little, E. L., Jr.; Brinkman, K. A.; McComb, A. L. *For. Sci.* **1957**, *3*, 253–262.
72. Goerndt, M. E.; Mize, C. *North. J. Appl. For.* **2008**, *25* (2), 82–86.
73. Green, B. K. M.S. Thesis, Iowa State University, Ames, IA, 1998, 63 p.
74. Hall, R. B.; Ruigu, S. In *Fifth International Poplar Symposium 2010*; Internatinal Union of Forestry Research Organizations (IUFRO): Viterbo, Italy, 2010, p 75.
75. Hall, R. B.; Colletti, J. P.; Schultz, R. C.; Faltonson, R. R.; Kolison, S. H., Jr.; Hanna, R. D.; Hillson, T. D.; Morrison, J. W. USDA Forest Service Gen. Tech. Rep. NC-GTR-140; 1989, pp 211−219.
76. Eaton, J. In *IEA Bioenergy Task 30 Workshop*; Guelph, Ontario, Canada, August 13, 2007.
77. Hall, R. B.; Hart, E. R.; Peszlen, I. In *Molecular Breeding of Woody Plants*; Morohoshi, N, Komamine, A., Eds.; Elsevier Science B.V.: 2001, pp 229−238.

Downloaded by STANFORD UNIV GREEN LIBR on June 8, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch002

78. Stanosz, G. R; Kruger, E. L.; Isebrands, J. G. In *BioEnergy '98: Expanding Bioenergy Partnerships*: Madison, WI, October 4−8, 1998.

79. Gruenewald, H.; Brandt, B. K. V.; Uwe Schneider, B.; Bens, O.; Kendzia, G.; Huttl, R. F. *Ecol. Eng.* **2007**, *29*, 319–328.

80. Zenone, T.; Migliavacca, M.; Montagnani, L.; Seufert, G.; Valentini, R. 2007. http://www.fao.org/forestry/13710-1-0.pdf (Last accessed on 11/16/10).

81. Hall, R. B.; Headlee, W.; Ruigu, S. In *Fifth International Poplar Symposium 2010*; Internatinal Union of Forestry Research Organizations (IUFRO): Viterbo, Italy, 2010, p 98.

82. Headlee, W. L.; Hall, R. B.; Zalesny, R. S., Jr. In *Fifth International Poplar Symposium 2010*; Internatinal Union of Forestry Research Organizations (IUFRO): Viterbo, Italy, 2010, p 129.

83. Hall, R. B. In *Proceedings of the 1993 Joint Meeting of the Poplar Councils of the United States and Canada*; Bronstein, R., Ed.; Poplar Council of Canada: 1993; pp 68−73.

84. Zsuffa, L.; Sennerby-Forsse, L.; Weisgerber, H.; Hall, R. B. In *Clonal Forestry II, Conservation and Application*; Ahuja, M. R., Libby, W. J., Eds.; Springer Verlag: Berlin, 1993; pp 91−119.

85. Börjesson, P. *Biomass Bioenergy* **1999**, *16*, 137–154.

86. Volk, T. A.; Abrahamson, L. P.; Nowak, C. A.; Smart, L. B.; Tharakan, P. J.; White, E. H. *Biomass Bioenergy* **2006**, *30*, 715–727.

87. Richardson, J.; Cooke, J. E. K.; Isebrands, J. G.; Thomas, B. R.; van Rees, K. C. J. *Can. J. Bot.* **2007**, *85*, 1136–1146.

88. Zsuffa, L. *Biomass* **1990**, *22*, 35–47.

89. Buchholz, T. S.; Volk, T. A. *BioEnergy Res.*, in press, DOI:10.1007/s12155-010-9103-5

90. Smart, L. B.; Cameron, K. D.; Volk, T. A.; Abrahamson, L. P. *NABC Report 19*; *Agricultural Biofuels*; National Agricultural Biotechnology Council: Ithaca, NY, 2008; pp 85−92.

91. Newsholme, C. *Willows, The Genus Salix*; Timber Press: Portland, OR, 1992; 224 p.

92. Kuzovkina, Y. A.; Weih, M.; Romero, M. A.; Charles, J.; Hurst, S.; McIvor, I.; Karp, A.; Trybush, S.; Labrecque, M.; Teodorescu, T. I.; Singh, N. B.; Smart, L. B.; Volk, T. A. In *Horticultural Reviews*; Janick, J., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, 2008; Vol. 34, pp 447−489.

93. Dickmann D. I.; Kuzovkina, J. In *Poplars and Willows in the World*; Isebrands, J. G., Richardson, J., Eds.; Working Paper IPC/9-2. FAO, Rome, Italy, 2008.

94. Smart, L. B.; Cameron, K. D. In *Genetic Improvement of Bioenergy Crops*; Vermerris, W., Ed.; Springer: 2008, pp 347−376.

95. Nordman, E. E.; Robison, D. J.; Abrahamson, L. P.; Volk, T. A. *For. Ecol. Manage.* **2005**, *217*, 307–318.

96. Hunter, T.; Royle, D. J.; Arnold, G. M. *Ann. Appl. Biol.* **1996**, *129*, 1–12.

97. Peacock, L.; Hunter, T.; Turner, H.; Brain, P. *J. Appl. Ecol.* **2001**, *38*, 1070–1081.

Downloaded by STANFORD UNIV GREEN LIBR on June 8, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch002

98. Collins, C. M.; Fellowes, M. D. E.; Sage, R. B.; Leather, S. R. *Agric., Ecosyst. Environ.* **2001**, *3*, 183–189.
99. Argus, G. W. *Systematic Botany Monographs*; American Society of Plant Taxonomists: Ann Arbor, MI, 1997; 121 p.
100. Argus, G. W. Botanical Electronic News (BEN) #227. 1999. http://www.ou.edu/cas/botany-micro/ben227.html (Last accessed on 11/16/10).
101. Lin, J. Z.; Zsuffa, L. *Silvae Genet.* **1993**, *42*, 126–131.
102. Cameron, K. D.; Phillips, I. S.; Kopp, R. F.; Volk, T. A.; Maynard, C. A.; Abrahamson, L. P.; Smart, L. B. *BioEnergy Res.* **2008**, *1*, 80–90.
103. Tharakan, P. J.; Volk, T. A.; Lindsey, C. A.; Abrahamson, L. P.; White, E. H. *Energy Policy* **2005**, *33*, 337–347.
104. Adegbidi, H. G.; Volk, T. A.; White, E. H.; Abrahamson, L. P.; Briggs, R. D.; Bickelhaupt, D. H. *Biomass Bioenergy* **2001**, *20*, 399–411.
105. Adegbidi, H. G.; Briggs, R. D.; Volk, T. A.; White, E. H.; Abrahamson, L. P. *Biomass Bioenergy* **2003**, *25*, 389–398.
106. Volk, T. A.; Kiernan, B. D.; Kopp, R.; Abrahamson, L. P. In *Fifth Biomass Conference of the Americas*; Orlando, FL, 2001.
107. Volk, T. A. Ph.D. Thesis, SUNY-ESF, Syracuse, NY, 2002.
108. Labrecque, M.; Teodorescu, T. I. *Biomass Bioenergy* **2005**, *29*, 1–9.
109. Volk, T. A.; Luzadis, V. In *Renewable Energy from Forest Resources in the United States*; Routledge Press: New York, 2009; pp 238−260.
110. Prestemon, J. P.; Abt, R. C.USDA Forest Service Gen. Tech. Rep SRS-GTR-53; 2002, pp 299−325.
111. Zhang, D.; Polyakov, M. *Biomass Bioenergy* **2010**, *34* (12), 1643–1654.
112. Stanturf, J. A.; Kellison, R. C.; Broerman, F. S.; Jones, S. B. *J. For.* **2003**, *101* (3), 26–31.
113. Stanturf, J. A.; Kellison, R. C.; Broerman, F. S.; Jones, S. B. *For. Policy Econ.* **2003**, *5* (4), 407–419.
114. Allen, H. L.; Fox, T. R.; Campbell, R. G. *South. J. Appl. For.* **2005**, *29* (2), 62–69.
115. Carter, M. C.; Foster, C. D. *For. Ecol. Manage.* **2006**, *227* (1-2), 137–144.
116. Fox, T. R.; Jokela, E. J.; Allen, H. L. *J. For.* **2007**, *105* (7), 337–347.
117. Jokela, E. J.; Dougherty, P. M.; Martin, T. A. *For. Ecol. Manage.* **2004**, *192* (1), 117–130.
118. McKeand, S.; Mullin, T.; Byram, T.; White, T. *J. For.* **2003**, *101* (3), 32–37.
119. Wear, D. N.; Greis, J. G. In *The Southern Forest Resource Assessment: Final Report*; USDA Forest Service, Southern Research Station: Asheville, NC, 2002; 675 p.
120. Wear, D. N.; Newman, D. H. *J. For.* **2004**, *102* (8), 25–31.
121. Wear, D. N.; Greis, J. G. *J. For.* **2002**, *100* (7), 6–15.
122. Butler, B. U.S. Forest Service, Northern Research Station, 2010; personal communication.
123. Skog, K. E.; Stanturf, J. A. Forest Biomass Sustainability and Availability. Zho, J. Y., Zhang, X., Pan, X., Eds.; ACS Syposium Series 1067; American Chemical Society: Washington, DC, 2011; Chapter 1.
124. Colbert, S. R.; Jokela, E. J.; Neary, D. G. *For. Sci.* **1990**, *36* (4), 995–1014.

Downloaded by STANFORD UNIV GREEN LIBR on June 8, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch002

125. Burkes, E. C.; Will, R. E.; Barron-Gafford, G. A.; Teskey, R. O.; Shiver, B. *For. Sci.* **2003**, *49* (2), 224–234.
126. Jokela, E. J.; Wilson, D. S.; Allen, J. E. *South. J. Appl. For.* **2000**, *24* (1), 23–30.
127. Borders, B. E.; Bailey, R. L. *South. J. Appl. For.* **2001**, *25*, 69–74.
128. Roth, B. E.; Jokela, E. J.; Martin, T. A.; Huber, D. A.; White, T. L. *For. Ecol. Manage.* **2007**, *238* (1−3), 175–188.
129. McKeand, S. E.; Jokela, E. J.; Huber, D. A.; Byram, T. D.; Allen, H. L.; Li, B.; Mullin, T. J. *For. Ecol. Manage.* **2006**, *227* (1−2), 178–184.
130. Berisford, C. W.; Kulman, H. M. *For. Sci.* **1967**, *13*, 428–438.
131. Nowak, J. T.; Berisford, C. W. *J. Econ. Entomol.* **2000**, *93* (2), 336–341.
132. Coyle, D. R.; Nowak, J. T.; Fettig, C. J. *J. Entomol. Sci.* **2003**, *38* (4), 621–630.
133. Cade, S. C.; Hedden, R. L. *South. J. Appl. For.* **1987**, *11*, 128–133.
134. Ross, D. W.; Berisford, C. W.; Godbee, J. F. *For. Sci.* **1990**, *36*, 1105–1118.
135. Nelson, C. D.; Kubisiak, T. L.; Amerson, H. V. *For. Pathol.* **2010**, *40* (1), 64–72.
136. Whetten, R. W.; Kellison, R. *J. For.* **2010**, *108* (4), 193–201.
137. Albaugh, T. J.; Lee Allen, H.; Dougherty, P. M.; Johnsen, K. H. *For. Ecol. Manage.* **2004**, *192* (1), 3–19.
138. Adegbidi, H. G.; Jokela, E. J.; Comerford, N. B. *For. Ecol. Manage.* **2005**, *218* (1-3), 245–258.
139. Scott, D. A.; Tiarks, A. *South. J. Appl. For.* **2008**, *32* (1), 33–37.
140. ArborGen FlexStand System Guide. http://www.arborgen.us/uploads/catalog/FlexStand_Brochure_v10.pdf (Last accessed on 11/16/10).
141. Catchlight Intercropping. http://www.catchlightenergy.com/Feedstocks.aspx (Last accessed on 11/16/10).
142. Sims, R. E. H.; Hastings, A.; Schlamadinger, B.; Taylor, G.; Smith, P. *Global Change Biol.* **2006**, *12* (11), 2054–2076.
143. Rockwood, D. L.; Kirst, M.; Isebrands, J. G.; Zhu, J. Y. In *Handbook of Bioenergy Crop Plants*; Kole, C., Joshi, C. P., Shonnard, D. R., Eds.; CRC Press, Taylor and Francis Group: 2011, in press.
144. Sims, R. E. H. *Mitigation Adapt. Strategies Global Change* **2003**, *8* (4), 349–370.
145. Wright, L. *Biomass Bioenergy* **2006**, *30*, 706–714.
146. Whitesell, C. D.; DeBell, D. S.; Schubert, T. H.; Strand, R. F.; Crabb, T. B. USDA Forest Service Gen. Tech. Rep PSW-GTR-137; Albany, CA, 1992; 30 p.
147. Forest Solutions. www.hawaiiforest.com/commercial_forestry.htm (Last accessed on 11/16/10).
148. Rockwood, D. L.; Rudie, A. W.; Ralph, S. A.; Zhu, J. Y.; Winandy, J. E. *Int. J. Mol. Sci.* **2008**, *9*, 1361–1378.
149. Langholtz, M.; Carter, D. R.; Rockwood, D. L.; Alavalapati, J. R. R. *J. For. Econ.* **2007**, *12*, 237–249.
150. Rockwood, D. L.; Tamang, B. *Proc. Florida State Hort. Soc.* 2010, (in press).
151. Stape, J. North Carolina State University, 2010; personal communication.

Downloaded by STANFORD UNIV GREEN LIBR on June 8, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch002

152. ArborGen. http://www.arborgen.us/uploads/publications/eucalyptus-plantations.pdf; http://www.arborgen.us/uploads/tree-lines-newsletters/TreeLines_Spring10_v05_1.pdf; http://www.arborgen.us/index.php/products/product-pipeline/freeze-tolerant-eucalyptus (Last accessed on 11/16/10).
153. Gonzalez, R.; Wright, J.; Saloni, D. *Biomass Mag.* **2010**, *41*, 52–55.
154. Hansen, E. A. *Biomass Bioenergy* **1993**, *5*, 431–436.
155. Isebrands, J. G.; Karnosky, D. F. In *Poplar Culture in North America*; Dickmann, D. I., Isebrands, J. G., Eckenwalder, J. G., Richardson, J., Eds.; NRC Research Press, National Research Council of Canada: Ottawa, ON, 2001; pp 207−218.
156. Moser, B. W.; Pipas, M. J.; Witmer, G. W.; Engeman, R. M. *Northwest Sci.* **2002**, *76*, 158–165.
157. Gladstone, W. T.; Ledig, F. T. *For. Ecol. Manage.* **1990**, *35*, 69–78.
158. Joslin, J. D.; Schoenholtz, S. H. *Biomass Bioenergy* **1997**, *13*, 301–311.
159. Johnson, J. M. F.; Coleman, M. D.; Gesch, R.; Jaradat, A.; Mitchell, R.; Reicosky, D.; Wilhelm, W. W. *Am. J. Plant Sci. Biotechnol.* **2007**, *1*, 1–28.
160. Hill, J.; Nelson, E.; Tilman, D.; Polasky, S.; Tiffany, D. *Proc. Nat. Acad. Sci. USA* **2006**, *103*, 11206–11210.
161. Powers, R. F. *New For.* **1999**, *17* (1), 263–306.
162. Janowiak, M. K.; Webster, C. R. *J. For.* **2010**, *108* (1), 16–23.
163. Brockerhoff, E.; Jactel, H.; Parrotta, J.; Quine, C.; Sayer, J. *Biodiversity Conserv.* **2008**, *17* (5), 925–951.
164. Carnus, J. M.; Parrotta, J.; Brockerhoff, E.; Arbez, M.; Jactel, H.; Kremer, A.; Lamb, D.; O'Hara, K.; Walters, B. *J. For.* **2006**, *104* (2), 65–77.
165. Vance, E. D. *For. Ecol. Manage.* **2000**, *138* (1−3), 369–396.
166. Shepard, J. P. *Biomass Bioenergy* **2006**, *30* (4), 378–384.
167. Lucier, A. A.; Shepard, J. P. *Biomass Bioenergy* **1997**, *13* (4-5), 193–199.
168. Runge, C. F.; Senauer, B. *Foreign Affairs* **2007**, *86* (3), 41–53.
169. Munsell, J. F.; Fox, T. R. *Biomass Bioenergy* **2010**, *34* (12), 1631–1642.
170. Zalesny, R. S., Jr.; Donner, D. M.; Coyle, D. R.; Headlee, W. L.; Hall, R. B. In *8th Biennial Short Rotation Woody Crops Operations Working Group Conference: Short Rotation Woody Crops in a Renewable Energy Future: Challenges and Opportunities*; Syracuse, NY, October 17−21, 2010.
171. Volk, T. A.; Verwijst, T.; Tharakan, P. J.; Abrahamson, L. P. *Front. Ecol. Environ.* **2004**, *2*, 411–418.
172. Rowe, R. L.; Street, N. R.; Taylor, G. *Renewable Sustainable Energy Rev.* **2008**, *13*, 271–290.
173. Ranney, J. W.; Mann, L. K. *Biomass Bioenergy* **1994**, *6*, 211–228.
174. Aronsson, P. G.; Bergstrom, L. F.; Elowson, S. N. E. *J. Environ. Manage.* **2000**, *58*, 135–45.
175. Tolbert, V. R.; Todd, V., Jr.; Mann, L. K.; Jawdy, C. M.; Mays, D. A.; Malik, R. *Environ. Pollut.* **2002**, *16*, S97–S106.
176. Ulzen-Appiah, F. Ph.D. Thesis, SUNY-ESF, Syracuse, NY, 2002.
177. Rosenqvist, H.; Dawson, W. M. *Biomass Bioenergy* **2005**, *29*, 83–92.
178. Heller, M. C.; Keoleian, G. A.; Volk, T. A. *Biomass Bioenergy* **2003**, *25*, 147–65.

Downloaded by STANFORD UNIV GREEN LIBR on June 8, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch002

179. Dhondt, A.; Wrege, P. H.; Cerretani, J.; Sydenstricker, K. *Bird Study* **2007**, *54*, 12–22.
180. Heller, M. C.; Keoleian, G. A.; Mann, M. K.; Volk, T. A. *Renewable Energy* **2004**, *29* (7), 1023–1042.
181. Keoleian, G. A.; Volk, T. A. *Crit. Rev. Plant Sci.* **2005**, *24*, 385–406.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch002

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Chapter 3

Biomass Logistics – Harvest and Storage

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The goal of biomass logistics is to provide low cost feedstocks with the desired physical and chemical properties; harvested, stored, and transported in a sustainable and economic manner. This chapter will concentrate on four types of biomass: forest material and residues, short-rotation coppice crops, perennial grasses and agricultural residues. No matter the type of biomass, the means of harvest and storage are most affected by the feedstock moisture and bulk density. Biomass less than 20% (w.b.) moisture can be stably stored under aerobic conditions for long periods provided they are protected from the precipitation. If biomass is stored under aerobic conditions above this moisture, there will be undesirable decomposition, loss of dry matter, and risk of fire from spontaneous combustion. Material stored at greater than 20% (w.b.) moisture is typically preserved either by ensiling or by applying additives or amendments that retard undesirable biological activity. The choice of harvesting system is influenced in great part by the moisture and density required of the feedstock.

Harvesting Conventional Forest Crops and Residues

Equipment used to harvest conventional forest crops are intended for use in rough, steep terrain and are designed to handle large trees so they have high up-front capital costs and significant operating costs (*1*). There are two major types of tree harvesters: feller/bunchers and cut-to-length harvesters. A feller/buncher is a wheel or track driven harvester with an articulated attachment that can cut and gather one or more trees before felling them into a bunch. The tree is severed from its base either by circular saw blades or a shear. Shears are somewhat slower

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than saws but they are low-cost and robust (*1*). The articulated gripping attachment places the cut tree on a stack suitable for a skidder or forwarder to transport the tree from the forest for further processing. Cut-to-length (CTL) harvesters are similar to feller/bunchers except that they also delimb the trunk, cut the trunk to specific length, and stack the cut logs. The stacks are then removed from the forest with a forwarder. Advantages of CTL harvesters include more residue/slash left in the forest, less forest damage because skidders are not used, and fewer operations (*1*).

Skidders and forwarders perform the same task as the CTL harvesters – removal of the cut trees from the forest to landing sites where they may be delimbed and cut-to-length. Skidders use a cable or grapple to drag the tree to the landing site. Forwarders use a grapple to collect the trees and place them in a transport cradle. Once the forwarder cradle is full, the load is transported to the landing site. Skidders are preferred over forwarders when the terrain will not allow forwarder use or if selective logging of larger timber is desired. Since forwarders carry logs in the transport cradle, they have much less impact on the forest floor but timbers must be limited to the size of the cradle.

Forest slash is essentially all the tree residue left on the forest floor or at the landing site after the cut tree has been delimbed, debarked and cut to length. Slash can serve as important cover for the forest floor, protecting it from erosion and serving as wildlife habitat. It is also a source of nutrients to replenish the forest soil. However, slash can be also a fuel source for forest fires after harvest is complete. Various harvesters are available that use an articulated grapple to capture slash. The slash is then compressed into bales or bundles that are transported from the forest to landing sites.

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Harvesting Short Rotation Coppice (SRC) Crops

The most frequently considered short rotation coppice crops (SRC) crops are willow (*Salix*), eucalyptus (*Eucalyptus*) and poplar (*Populus*). Others species include included fast growing species in sycamore (*Platanus*), sweetgum (*Liquidambar*) and pine (*Pinus*) genera. To maximize field efficiency during harvest, it is desirable to plant long straight rows so that time spent during turns becomes a small fraction of the total operation time. Headlands, i.e. areas not planted to SRC at the field ends, are recommended to be 4 to 5 m wide to facilitate turning harvest equipment (*2*). Headlands can be damaged during winter harvests, so they are typically planted to grass (*3*). SRC can be planted in single rows with 1 m spacing between and in the rows or in twin-rows with pairs of rows 0.75 m apart and 1.5 m spacing between pairs with 0.6 m spacing between trees in the row (*2*). Twin rows are more popular because this scheme provides the desired plant density (15,000 to 20,000 plants/ha) with more clearance for harvesting equipment. The wide row spacing also helps prevent branch entanglement which would make harvesting adjacent rows more difficult.

When SRC are severed from their root system, the cutting tool encounters several stems of various diameters. Although stems tend to grow vertically from the stump, some stems form an elbow between the stump and their vertical projection (*4*). Cutting at the elbow requires more energy because of the larger cutting area; so cutting height is typically 100 to 150 mm off the ground to avoid elbows. This cutting height also insures that the stems are separated and damage to the stump is prevented.

Harvesting generally takes place on a three or four year cycle with the first harvest three years after cutback. Costs per unit mass were 12% less with a four-year cycle (*5*) because fixed costs are diluted by greater yield; however cash flow is more challenging with the longer harvest cycle. Harvest typically takes place in the period between leaf senescence in the fall and before bud-break in the spring. Budding cause's movement of nutrient reserves from the roots and stems to the developing shoots. Harvest after budding can weaken the plant, reducing vigor, stand life and yield (*2*, *6*). Depending upon location, harvest usually takes place from mid-October to early March. Snow cover or soil firmness often limit when harvest can occur during this period. Wheel traffic in wet plantations, particularly during winter harvest, can lead to soil compaction and rutting which reduces long-term productivity of the stand (*2*).

Compared to conventional forests, SRC plantations are relatively flat, obstacle-free with small trees of uniform size growing in straight, uniformly spaced rows. The trees have relatively small branches and bark characteristics compared to typical conifers, all of which suggest that biomass grown in SRC plantations can be harvested, processed and transported more cost effectively by using other than conventional forestry equipment (*1*).

Severing the stems from the stump is typically done with circular saws, intermeshing discs, chain saws, inertial single blade, or horizontal flails (*4*). Circular saw blades are most commonly used because they produce the cleanest cut and require less maintenance than other technologies. Additionally, intermeshing discs, inertial blades and flails all produce a ragged cut that can negatively affect regrowth (*4*). The tip speed of the cutting blade should range from 10 to 70 m/s (*7*). Higher speeds produce a cleaner cut but require more energy.

SRC are typically harvested in three ways: rods/sticks/whole-plants; billets; and chips. SRC are harvested when the plants are dormant but still growing, so they typically are 45 – 65% (w.b.) moisture at harvest (*6*, *8*, *9*). Depending upon the end-use, the feedstock moisture will most likely need to be reduced during storage and before use. The type of harvest practiced can affect the rate of feedstock drying after harvest. Rods or sticks can be piled in the field allowing air flow through the loose pile to facilitate drying. The density of rod or stick bundles is 0.1 Mg DM/m^3 (*10*). Billets (60 – 200 mm) make a more dense pile (0.13 – 0.17 Mg DM/m^3) but still allow enough air flow through the pile so that natural drying takes place (*10*). Wood chips stored outdoors in piles can suffer from losses of substrate due to biological activity, causing subsequent reduction in product yield. Substrate losses vary depending on wood species, ambient conditions, precipitation, pile size and shape, presence of fines, and storage duration. When SRC are chipped and piled, respiration of plant cells and microbial activity consume substrate and release energy causing a rise in pile temperature (*11–15*). The rise in pile temperature can be attributed to two phenomena. First, the small particle-size of the chips and the presence of fines limit airflow. Second, because

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wood has low thermal conductivity. The result is that temperatures in excess of 70°C as well as the incidence of spontaneous combustion has been observed (*16*).

No matter the type of harvest used, end-users will eventually require the fuel in chip form, typically 8 – 35 mm depending upon intended use of the chips. The distribution of chip particle- size influences the handling, bridging, ventilation, and combustion characteristics of solid particulate biofuels (*17*). Bridging problems increase with long, thin particles (*18*). Wood chips are typically size classified by mechanical screening using a cascade of sieves with progressively smaller openings to sort the particles by decreasing size. Size classification techniques using mechanical sieving are standardized (*19*)

With rod/stick/whole-plant harvesting, the SRC stems are cut at the base and gathered by the harvester. These harvesters are lower cost than billet and chip harvesters. Additionally, rod or sticks dry naturally with reasonably low dry matter loss. However, rods or stick bundles occupy more space, are harder to handle and transport than billets or chips, and have additional costs associated with secondary handling and chipping operations (*2*). Rod or stick harvesters typically harvest one or two rows at a time and use a saw blade to cut the plant. These machines do no size reduction. The plants are grouped into bundles that weigh up to 1.5 Mg that are dropped at any location in the field. Ideally the bundles would be dropped at the headlands, but bundle mass, yield and field size all dictate where bundles are dropped. Bundles may be left in the field until it is convenient to collect them or they may be gathered soon after harvest. Rod or stick bundle harvesters have an added feature where the whole stems are cut into lengths of 1.2 – 2.5 m and then wrapped in polypropylene mesh to form a tight bundle. After a period of field drying, the bundles are loaded onto wagons or trucks using grapples, and are then transported to the storage area, where they are stacked and stored. Whole-plant harvesters are less productive than billet or chip harvesters because typically the machine must stop harvest each time a bundle is formed and dropped (*20*).

Billet harvesters offer an intermediate step between the rod/stick harvesters and single-pass chip harvesters. Billet harvesting is a continuous process which creates a product that can be more easily handled than rods/sticks, but post-storage chipping is still required. Billet harvesters use a saw blade to cut whole stems from the base, and then cut the stems into billets that are typically 150 to 200 mm in length. Many coppice billet harvesters are modified sugar cane harvesters with saw blades adapted to the head to facilitate cutting. After cutting from the root, the stem is fed butt-end first into a set of meshing cutting drums that size-reduce the stem into billets. The number of blades on the cutting drums dictates the billet length. Typically either 3 or 4 knives are used on each cutting drum. If a four knife drum is used, billet length can be doubled when two knives are removed. The plant material is then passed through a cleaning system where free bark, leaves and trash are blown from the billet stream and deposited on the ground. Billets are conveyed to a wagon or truck operating alongside the harvester. Billet harvesting is a continuous process with outputs of up to 30 Mg DM per h reported (*10*).

Whole-plant chip harvesters are typically self-propelled forage harvesters that have been converted for harvesting coppice plants. Forage harvesters are widely used throughout the world to harvest animal feed, they have large harvesting capacity, and are very adoptable so their substantial costs can be diluted over

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many crops. The only modification typically made to convert a forage harvester to harvest SRC is a new crop gathering unit. Modified crop gathering units typically use high-speed saw blades, slow-speed feeding towers, and butt lift paddles similar to those used on billet harvesters. There is a forward mounted bar that tips the top of the plant forward so that the stem is fed into the machine butt-end first. Butt end feeding is critical to insure smooth material flow into the feedrolls. The feedrolls gather, compress and meter the stems into the cutterhead in a controlled manner. The crop is size-reduced by a multi-knife cylindrical cutterhead working in conjunction with a stationary shearbar. The chip size can be changed on-the-go by altering the relative speed of the feedrolls with respect to the cutterhead speed. Removing knives from the cutterhead can further increase chip size. The typical chip size is 10 to 45 mm with a log-normal distribution. The chipped SRC is conveyed from the harvester by an impeller/blower into a wagon or truck operating alongside the harvester. Currently, SRC can only be harvested in chipped form two-rows at a time. Outputs range from 16 to 24 Mg DM/h depending upon yield and power availability. This productivity is much less than the same harvester would be capable of when harvesting whole-plant corn silage, where instantaneous throughputs can exceed 250 fresh Mg/h or 80 MG DM/h (*21*). The productivity difference can be attributed to differences in the shear energy between corn and SRC and the difficulties of smoothly feeding the tall SRC stems into the feedrolls. The diesel fuel consumption for harvesting SRC ranges from 1.0 to 1.3 L/Mg (as harvested) for billet or chip harvesters and 0.5 to 1.0 L/Mg for whole-plant harvesters (*4*).

Most billet or chip harvesters require that the end product be transported in bulk and piled for storage. An alternative approach is to size-reduce in the field and place the product into a bundle that has a higher density for better transport efficiency and a smaller footprint for storage. An agricultural round baler is available which size-reduces and packages SRC crops (*22*). Modifications included the addition of a more maneuverable hitch; a bank of four rotary saw blades followed by hammer-shredder to break the stems and make them more pliable; and finally full-width compression belt. Harvester/baler capacity was 5 to 9 wet Mg/h with bales 0.99 to 1.54 m diameter. Average moisture content of the bales was 44% to 51% with bale density of 111 to 167 kg DM/m^3 (*22*). The bale density was slightly greater than that reported for chipped SRC (table 1).

Harvesting Perennial Grasses

Perennial grasses that are considered good candidates for biomass feedstocks include switchgrass (*Panicum virgatum*), reed canarygrass (Phalaris arundinacea), indiangrass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*) , and miscanthus (*Miscanthus giganteus*). Perennial grasses will typically be harvested only once per year, either in the fall after senesce or in the spring right before the next growing cycle. Although yield is often greater using a two-cut or three-cut system, yield on the subsequent harvests are not great enough to justify the added harvesting costs (*23–26*). Also, if more than two cuttings per year are removed, additional N might need to be applied to compensate for the N removed in the late-season harvest. In a single-cut system, fall harvest of reed canarygrass and switchgrass reduced yields by 2–5% and 1–14%, respectively, compared to a single summer cutting (*27*). When these grasses were left standing over winter and harvested in the spring, DM yields were reduced by 17–27% (*27–29*) because the plants had senesced with fallenleaves decomposed and lost. . However, spring harvest would allow baling directly at cutting because average the average moisture content is often well below 15% (w.b.) (*27*).

Perennial grass biomass feedstocks are typically harvested as a field dried product packaged in bales and then stored under aerobic conditions. To prevent biological degradation, the aerobically stored bales must be harvested below 20% (w.b.) moisture. It is not practical or economically feasible to dry biomass other than by field drying. Grass drying rate is affected by environmental factors such as solar intensity, relative humidity, and soil moisture; swath density; and type of crop (*30*). The drying rate of switchgrass and reed canarygrass were greater than those reported for forage crops despite the fact that the feedstock grasses had yields over twice that of typical forages (*27*). This was because the biomass grasses were drier at cutting and formed a well-formed swath structure which allowed good air movement through the swath. Biomass grass drying rate is enhanced by cracking the stem to provide an egress for stem moisture and by spreading the swath across the full cut-width to maximize solar insolation (*27*).

Baling productivity is dependent upon many factors including available power, yield, crop moisture, machine capacity, and system efficiency. Baling productivity is 8.3 to 17.7 and 21.6 to 25.2 Mg DM/h with a large round and large square baler, respectively (*27*). Round baler productivity is less because baling has to be interrupted during the wrapping process while square baling is a continuous process. Bale density is important because it impacts the area required for storage and transportation and handling costs. Density of perennial grasses in large round and square bales varies from 140 to 170 kg DM/m^3 and 180 to 200 kg DM/m^3, respectively (*27*, *31*, *32*).

Perennial grasses can be harvested at moistures above 20% (w.b.) moisture provided they are stored anaerobically and preserved be fermentation or by application of additives to rapidly drop the pH. Typically harvest involves a period of field wilting to the desired moisture followed by harvesting with either a baler or a precision-cut forage harvester. The main advantage of this harvest scheme is that the field wilting period is shortened and weather related harvest delays are reduced, leading to a more robust harvest system. Harvest by direct-cutting and chopping with the forage harvester can eliminate the cutting and field wilting operations provided the standing moisture of the crop appropriate for anaerobic storage.

Harvesting Crop Residues

Crop residues that are considered good candidates for biomass feedstocks are generally limited to corn (*Zea mays L.*) stover or small grain straw. Stover or straw include all the above ground, non-grain fractions of these crops. With

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Table 1. Approximate densities of SRC after harvest

Package	***Density*** *kg DM/m³*
Rods/Sticks[1]	100
Billets[1]	125 – 165
Chips[1]	125 – 175
Bales[2]	110 – 170

[1] ref (*10*). [2] ref (*22*).

approximately 32 million ha of corn grown in the United States (*33*), the stover fraction is widely acknowledged as a leading feedstock candidate (*34*).

Corn stover is widely harvested today, mainly as high-fiber roughage feed for ruminant animals or as animal bedding. Stover is harvested as a dry product and packaged in large round or large square bales, typically involving as many as seven steps after grain harvesting (*35*). Problems with this system include slow field drying, short harvesting window, frequent weather delays, soil contamination, and low efficiency of cob collection. The cobs are the most valuable fraction of the stover because of their density and high energy content.

A single-pass harvesting system which produces corn grain and stover harvested in separate crop streams has been developed to eliminate subsequent field operations and reduce costs. Researchers have developed single-pass corn harvesters in which the grain and non-grain fractions were separated, processed and transported from the machine in separate streams (*36–40*). Single-pass harvesting significantly reduces total harvesting costs (grain plus stover) compared to harvesting dry stover in bales because of the eliminated harvesting steps (*21*). Single-pass harvesters size-reduce and collect all the non-grain material exiting the rear of the harvester. In addition to the elimination of all but one harvest operation, the attributes of this system included high stover yield, especially of the valuable cob, and low ash content. However, single-pass harvesters have some notable shortcomings. These included reduction in grain harvesting rate, high power consumption, insufficient stover size-reduction, low stover bulk density, and inability to manipulate the stover moisture by field curing. Finally, efficient field logistics are challenged by the need to simultaneously handle and transport stover and grain.

An alternative stover harvest system has been proposed that maintains many of the attributes of the single-pass system while overcoming many of its deficiencies. This two-pass system involves modifications to the combine to create a windrow of stover at the time of grain harvest (*21*, *40*, *41*). After grain harvest, the stover windrow can be harvested in a second pass using a forage harvester or baler. This harvesting system decouples grain and stover harvest, eliminating most of the logistic problems of simultaneous harvest. The system also requires fewer modifications to the combine harvester, and does not appreciably slow the rate of grain harvest (*40*). Finally, it allows for the manipulation of stover moisture so that either wet, ensiled storage or harvest and storage in dry bales are possible.

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If small grain straw will be harvested as biomass, it will be windrowed as the non-grain fraction exits the combine harvester. Yield of straw is increased by cutting the plant lower and ingesting more straw into the harvester. However, this tactic slows the harvest rate and reduces grain cleanliness and quality. The windrowed straw is often dry enough at grain harvest that it can be baled almost immediately, although some field drying may be required in humid climates. Straw is typically baled in large round or square bales. A stubble cutting attachment has been added to a rice cutting platform so that a majority of the straw was cut and windrowed without ingesting the straw into the harvester, thereby increasing combine capacity as well as straw yield (*42*). There has also been development of small grain harvesters in which the grain and chaff are harvested together and then separated post-harvest with the chaff used as biomass (*43*, *44*). Although these are single-pass systems, the majority of the small grain straw is left in the field so yield of biomass is low. These systems have seen limited commercial adoption.

Storage of Woody Biomass

Long-term storage of woody biomass is necessary for continuous operation at the conversion facility by allowing a supply buffer between harvest and utilization at the plant. Some storage can also allow the biomass to further dry increasing the usable energy content if used in a combustion process. However, long-term storage increases biomass cost due to storage facilities and additional handling. Additionally, biomass can be lost during storage due to volatilization and biological action from microorganisms and cause safety concerns with respect to respiration and spontaneous combustion. Most wood processing operations have significant investments in their wood supply and dedicate significant resources into proper biomass storage to insure safe and profitable operations.

Strategies to store biomass differ greatly depending on where the storage is located. Harvest-site storage is utilized to minimize storage needed at the processing plant, but requires additional logistics to track and retrieve. On-site storage allows for meeting the needs of a continuous operation and typically has biomass in communited form for easier handling. Densified forms such as pellets or briquettes represent a storage option typically done to reduce transport and storage costs and these forms have special storage considerations.

Harvest-Site Storage

Woody materials can typically be harvested year round with breaks only for weather conditions and times that ground conditions do no allow heavy equipment such as during the spring melt. Storage of woody materials “on the stump” is a great advantage over herbaceous crops because woody materials can be stored in place and harvested throughout the year. Once woody biomass is harvested one may either transport the material to the processing facility for storage or store immediately on-site. Advantages for on-site storage include decoupling the harvesting and processing operations, and providing a low cost storage option. Disadvantages include the inability to fully replant until the piles of biomass have been removed, and the cost of having to return to the site to collect and transport the material to the processing facility.

Harvest-site storage of pulpwood or timber grade logs is typically done immediately after harvest by creating uniform length logs and piling in a manner to facilitate easy loading onto logging trucks. In North America, non-lumber grade wood is typically cut to lengths of 2.4 m (8 ft.) to create cords which measure 1.22 x 1.22 x 2.4 m (4 x 4 x 8 ft.). The log piles are not typically covered as their density and shape limit infiltration from precipitation. Storage above the moist ground allows for transpiration induced drying which fosters the goal of partially drying the material. Dryer material has increased heating value if used for combustion and decreased mass for transportation. Logs can be seasoned in this fashion typically for 6 to 12 months depending on the region. Seasoning allows logs to reduce moisture content without utilizing purchased energy for drying (*45*).

The moisture content of biomass fuels can be expressed either on a wet basis ($M_{w.b.}$) or dry basis ($M_{d.b.}$).) (equations 1.1 and 1.2).

$$M_{w.b.}(wt\%) = \frac{mass_{water}}{mass_{water} + mass_{biomass}} \times 100\% \qquad \text{(Eq 1.1)}$$

$$M_{d.b.}(wt\%) = \frac{mass_{water}}{mass_{biomass}} \times 100\% \qquad \text{(Eq 1.2)}$$

The normal procedures for measuring wood moisture content are is to place small representative samples in an oven for 4 to 16 hours at 105°C to create an oven dry sample, which represents the dry mass of the biomass. The difference between the weight of the initial sample and the oven dry sample represents the sample water mass (*46*, *47*).

Green or freshly harvested wood has moisture content of approximately 50% (w.b.) meaning that for every kilogram of biomass there is a kilogram of water. Seasoned wood, that has been allowed to dry naturally for a year has moisture content of approximately 25% (w.b.) depending on weather and region. Wood is hygroscopic, meaning that it has an affinity for moisture and will have a moisture content in equilibrium with the humidity in the air. Wood dried under cover will dry to a moisture content of 6 to15% (w.b.) depending on the air temperature and relative humidity. Further drying by an oven or kiln drying is capable of reducing the biomass moisture to below 5% but the material will regain moisture upon reintroduction with humid air (*48*).

Logging residuals, including small diameter logs and branches are capable of being sustainably removed from the harvest. These materials can be stored on site with the logs to allow seasoning. The preferred system is to store the branches in unconsolidated mound-like piles of approximately 3 m wide and 2 m height or less. The piles should be located in an open, well-ventilated place to promote natural drying (*14*). The pile's leaves and needles are allowed to fall, thus reducing the material's ash content and recycling valuable nutrients to the soil (*49*).

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Processing-Site Storage

Mills and other wood processing facilities store logs, chip, or sawdust piles on-site or at satellite storage lots for times when it is not possible to deliver materials from the forest. The ideal storage period is determined by each facility's wood supply situation and cost of halting operations from lack of feedstock supply. Typical operations have 1- 2 months of on-site storage due to space limitations and inventory costs. Woody biomass is reduced in size in the forest and then transported for storage, or it is transported, reduced in size at the mill, and then stored. The resulting material, usually chips, is stored primarily outside in large piles but sometimes is stored under cover in large silos or bins. Chips stored in silos and bins are typically to be used within several hours or days while piles are used for longer-term storage needs. Silos and bins allow for uniform feeding and metering of the material. Many operations will have newly arrived materials from the forest directly feed the bins reserving piles for times of over or under delivery.

Chipping wood can be done with disc chippers or chippers to achieve particle sizes of 5 to 50 cm. Tub grinders or hammermills are used to achieve particle sizes below 5 cm, but at considerably greater energy investment. Disc chippers consist of a heavy rotating disc with two to eight radially protruding knives. The logs are fed into the disk and the knives shear the log by inertia with the log supported by an anvil plate. Particle-size is altered by adding or removing knives which alters knife spacing. Drum chippers consist of a cylindrical drum with embedded knives on the surface. Wood material is fed perpendicular to the longitudinal axis of the drum and the knives shear the wood by inertia. The log is supported by an anvil plate. Since the knives are on a curved surface instead of a flat disc, the angle changes with differing diameter logs being fed into it, creating higher variability in the cutting angle and resulting chip dimensions (*47*).

Storing size-reduced biomass facilitates mechanized handling and transport because conveyors and screw feeders can be employed. However, in storage the increased bulk density of the communited biomass can result in excessive microbiological activity. The increased susceptibility to heating is attributed to both low heat dissipation as well as anaerobic conditions. Additionally, chip piles can have excessive mold and fungi spores, which can cause health risk for humans as well as dry matter loss and quality and processing issues (*50*).

Wood chip piles that are below roughly 30% moisture (w.b.) have little issues with microbial decay. However green chipped material stored in a pile can experience rapid temperature rise due to cell respiration and aerobic microbes. Temperatures can rise in piles in the first several days up to 45°C, depending on such factors as moisture content, pile aeration, and initial microbial inoculation. Once oxygen is depleted, anaerobic bacteria and fungi thrive further heating the pile as high as 70°C. When these temperatures are reached, acetyl groups are liberated from hemicellulose creating acetic acid, which further increases the pile temperature and decreases the pH. If temperatures become high enough and pH low enough, the sugars in the wood undergoes acidolysis reactions which darken the wood. This process creates additional heat, raising chip piles temperatures above 105°C where pyrolysis can begin to occur. Although most bacteria and fungi can no longer function at these temperatures, the exothermic pyrolysis

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reactions can continue leading to spontaneous combustion especially if some oxygen is present. A time temperature depiction of deterioration for three chip piles is shown in Figure 1 (*14*, *51*).

High temperatures within the pile and acetic acid odor are signs that a chip or sawdust pile is experiencing undesired DM loss and has the risk of self-igniting. It is recommended that chip pile temperatures be monitored daily at many locations with thermocouples placed 0.5 m or more into the pile.

Tips for storing chipped materials to lower risk includes maintaining pile height below 15 m, minimizing compaction of the pile from heavy equipment, and preventing layering of particles caused by mixing fine particles such as sawdust or shavings with chips. It is also recommended to avoiding mixing biomass sources with different deterioration rates. In general hardwood deteriorate faster than softwoods, full-tree chipped biomass deteriorate faster than chips from stems that contain bark and foliage, and higher moisture biomass deteriorate faster than dryer biomass (*14*).

Densified Biomass Storage

Wood chips are easily produced and conveyed but have a relatively low dry bulk density of 150 to 200 kg/m^3. However, it is possible to further densify the biomass up to a dry bulk density of 600 kg/m^3 as a means to improve the storage and handling characteristics (*52*). Additionally, this processing step can provide improved consistency which will be beneficial for downstream processing. The typical densification process involves the following steps: milling, drying, densification, cooling, and transportation (*53*). All these steps are not necessary depending on the initial and desired end characteristics of the feedstock.

Milling of feedstock is performed to achieve smaller, more uniform particles. The smaller particle size helps create a more durable, high-density pellet. Although the finer the particle size the longer it will take to mill the feedstock and the more energy expended. Milling may not be necessary in certain waste wood applications where wood shavings and sawdust may be of appropriate particle size (*54*).

Drying typically follows the milling process because it is easier to dry the smaller material due to the greater exposed surface area. Drying the biomass prevents decomposition and allows for long term storage. Oven drying and moisture addition is used to achieve the appropriate moisture content for densification typically at around 10-12% moisture (w.b.) (*53*, *54*). Drying is important because moisture in the material acts as a lubricant thereby reducing the friction of material through the pellet die. Friction has many benefits in the pelleting process. First, it creates the backpressure that is required to consolidate the material. Second, friction resistance raises the temperature of the feedstock so that the glass fusion temperature of the natural binders in the material can be reached (*54*).

Biomass densification can occur with a variety of processes, which may be classified into three types: extrusion, roll briquetting, and pelletizing (*54*). Extrusion pelletizing forces the biomass through a fixed position die; roll briquetting compresses the biomass between two counter rotating rollers; and

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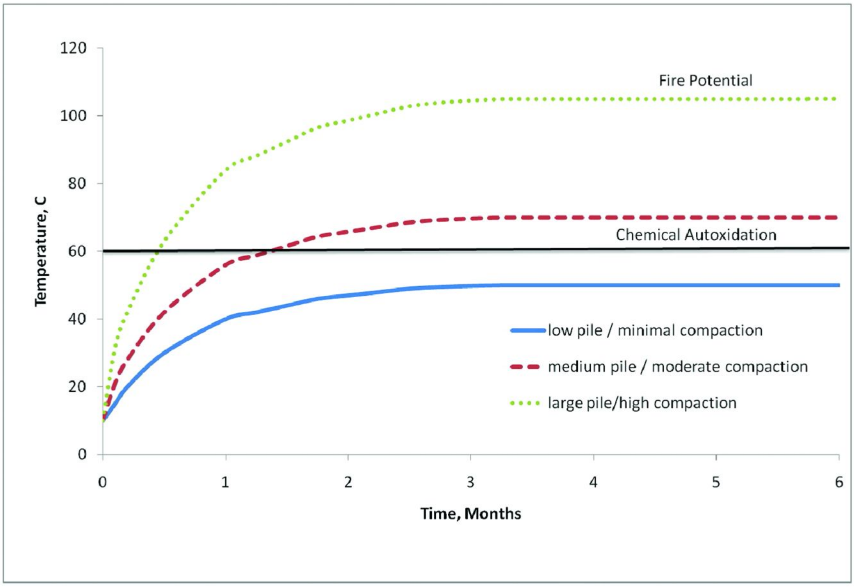

Figure 1. Chip pile temperature over the storage period (after (14, 51)).

pelletizing forces the biomass through a rotating external cylindrical die with rotating internal rollers (*52*, *53*). Pressure, temperature, and residence time are the typical processing parameters that influence the pellet density and quality. Feedstock characteristics of moisture and particle size are also critical to quality and are tightly controlled prior to the densification step (*55*).

High density, low moisture densified biomass are normally mechanically stable provided the biomass remains moisture free. However, when rehydrated, biomass pellets and briquettes lose form, creating the need to store pellets or briquettes under cover. (*52*, *56*). Pellets or briquettes are naturally hydrophilic and require storage to prevent rain from degrading durability and reducing heating values (*57*). Additionally densifed biomass will gain moisture from humidity with binderless products losing integrity above 93% humidity (*58*, *59*). Plastic or starch binders can be employed if higher stability is required, but with added costs (*55*, *60*). Factors that affect the choice of binder are cost, die wear characteristics, effect on strength and durability, density, and moisture uptake (*54*).

Care must be given to ventilate wood pellets during the storage and shipping of the materials in confined spaces. Biomass, even when dried, will decompose over time through chemical and biological means releasing CO, CO_2, and CH_4 which can become toxic at high concentrations (*61*). Additionally, immediately following the pelletization process, pellets can retain significant heat and moisture which can accelerate this deterioration. Aeration of bulk pellets is recommended to remove any gaseous products formed as well as lower moisture and temperature. Storage at the conversion facility should be limited to only a few days operation to limit costs and fire concerns in pellet piles (*62*).

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Storage of Herbaceous Biomass

There is considerable interest in storing agricultural residues and herbaceous perennials as feedstocks for bio-based chemicals, fuels and fibers (*34*). The logistics of storing and transporting the immense quantities of biomass needed to supply cost-competitive biorefineries is a great challenge. In addition to the large amounts of needed biomass, the low bulk density of such materials would preclude storage at the conversion facility. Storage at the conversion facility would be limited to several days needs to limit costs and fire concerns .

On-Farm Storage

Typically, on-farm residues and forages are stored wet (45 - 65 % w.b.) or dry (8 - 20 % w.b.). Dry biomass may be stored outdoors, indoors or under cover depending on the weather condition at the storage location and the allowable losses. On the other hand, the wet biomass must be stored anaerobically to prevent degradation.

Aerobic Storage

Dry, aerobic storage and transport has many advantages when compared to wet storage. Specifically, reducing overall transportation weight by decreasing the water fraction of the biomass allows increased transport distances. Consequently, a biorefinery could be operated at a larger scale as it could economically draw feedstock from a larger production area to one central location. This system is currently utilized in the production of dry hay in the Southwestern United States (U.S.) where conditions are favorable for harvest and outdoor storage, and the subsequent dissemination of that hay to the Midwest and Northeast.

Successful storage of dry biomass depends on primarily the moisture content, form (e.g., round bale, large square bale, chopped) and the storage environment (type and climate). Perennial grasses, legumes, and cereal grain corn and soybean residues are conventionally harvested in the form of round bales or large square bales. Additionally, crop residues can be dry precision chopped or stacked (loafed). However, chopped and loafed biomasses are mainly utilized on the farm and shipping distances are limited because of their low bulk density (*56*).

Depending on the type of biomass and form, the density can vary greatly (table 2). Here corn stover is on the low end of the range because its large-diameter stalks introduce a significant amount of porosity into the bale matrix, greatly reducing the overall density. Perennial grass (e.g., switchgrass, reed canarygrass) and legume (e.g., alfalfa) bales have densities near the high end of the range presented. Designers typically limit bale density to 240 (kg DM)/m^3 because this density maximizes legal shipping weight and volume of over the road trucks.

The storage form of biomass largely depends on the environmental conditions. In the Midwest and Northeastern U.S. outdoor storage is limited to round bale forms of cereal grain and corn residues, and low value hays. Round bales of biomass form a thatch on the outside layer that sheds precipitation and preserves DM in the core of the bale (*27*, *64*). Biomass in the large square bale form must be stored in-doors or under cover to prevent substantial DM losses from precipitation entering the layers of the bale. Dry matter losses for round bales stored outdoors can range from 4 to almost 30 percent of dry matter (% DM) for corn stover and between 5 and 15% DM for reed canarygrass and switchgrass (*27*, *63*). Indoor or covered storage can reduce dry matter losses to around 1 to 6%, but at a substantial storage cost (*27*, *64*, *66*, *67*). Tarps and plastic films have been employed to avoid the need for a storage structure whose primary purpose is to reduce exposure to moisture from rain events. More recently breathable films have been shown to produce dry matter losses similar to that of plastic films but are not yet commercially available (*27*).

Dry matter losses for outdoor-stored bales also depend on the exposure to the ground. Numerous studies have demonstrated that dry matter losses for bales can be significantly lower if stored off the ground or more practically, on a well-drained surface (*27*, *63*, *64*, *67*). Consequently, the storage environment plays a major role in dry matter loss. The data presented in these references represents outdoor storage in Wisconsin, Michigan and Oklahoma. However, it is assumed that losses would be lower in drier climates as rainfall has a great effect on dry matter loss. This fact is evident in the Southwestern U.S where even high value crops such as alfalfa are commonly stored outdoors in both round and large square bale forms.

Changes in crop composition during dry storage have also been documented, but vary by crop and storage situation and are beyond the scope of this text. Most research focuses on loss in quality parameters related to animal nutrition, which may or may not be of consequence for bio-chemical or thermal processes. For references see (*27*, *63*, *68*).

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Anaerobic Storage

Anaerobic (wet storage) methods have many advantages over a dry storage system, including low risk of fire, reduced harvesting costs, lower dry matter (DM) losses during storage, increased product uniformity, and improved feedstock susceptibility to enzymatic hydrolysis. Anaerobically-stored biomass can be kept in both baled (baleage) and chopped (silage) forms. Baled forms include round bales and large square bales. Bales are usually harvested after field wilting to moistures between 40 and 55% w.b. whereas chopped forms range from 35 to 67% depending on the storage structure (e.g., bags, bunkers, piles).

The long-term stability of anaerobically-stored silages depends upon ensuring appropriate amounts of readily-fermented carbohydrates, moisture content, and acidity (*69*). Normally, lactic acid bacteria (LAB) should ferment some of the silage's soluble sugars to lactic acid, thereby acidifying the forages and preventing further microbial activity during storage. In forages where moisture content is sufficiently low, fermentation of carbohydrates by LAB is favored over clostridia. However, if the biomass is too dry, LAB will also not grow.

Producers achieve the anaerobic environment favorable to LAB though a multitude of storage structures including tower silos, oxygen-limiting towers, plastic bags and film, in and above ground bunkers and covered piles. However, not all of these structures will be suitable to a low-value, high-volume commodity such as biomass. It is likely that biomass storage will be limited to large diameter bags, bunkers and piles.

Chopped crop stored in piles, bags and bunkers have many similarities. Each system involves densification of the biomass during when the crop is placed into storage to expel oxygen so as to achieve anaerobic conditions favorable for the afore-mentioned fermentation process and to prevent oxygen infiltration during the feed out period. Additionally, dry matter loss can be significant with the reintroduction of oxygen and moisture. Therefore the success of anaerobic systems depends on careful management throughout filling, storage and removal.

In a bag storage system, densification occurs by a bagger or silo press. Bag diameters vary from 2.4 m to 4.3 m and each diameter requires a specific machine. This machine uses a rotor to force the crop into a plastic bag constrained by a backstop. The backstop is connected to the bagging machine by heavy cables. The resistance to the spooling out of these cables is created by a hydraulic or manually actuated disk brake on the spool. As more of the crop is introduced to the bagger, the forces from the incoming silage exceed the braking force and cable is dispensed from the spool. The operator can manage bag density by means of this braking system. Density is estimated during the process by monitoring the circumferential elongation of the bag. Densities for silo bags range from 160 to 270 (kg DM)/)m^3 (*70*).

Density for piles and bunkers is managed by compressing the crop with the weight from tractors and in some cases field transports. The effective packing capacity of the system is a function of the thickness to which the incoming crop layer is spread, the packing tractor weight and the amount of time spent packing for each ton of crop (*70*). Therefore it is important to ensure the packing capacity is matched to harvesting productivity. Bunker silo densities range from 106 to 434 (kg DM)/)m^3 (*70*).

To ensure rapid fermentation and minimal dry matter loss, piles, bunkers and bags must be sealed with a barrier to oxygen and moisture. Polyethene commonly known as polyethylene is the most common material used for this purpose. In the case of a silo bag, a 150 μm polyethene tube encompasses the crop on all sides. The ends are sealed by tying the bag with a nylon string and/or covering with soil or gravel to prevent oxygen infiltration. Storage losses in bag silos depend on the feedstock and moisture content but have been observed to be around 5 to 10% of stored DM (*71*); however lower numbers have been presented in research trials of low moisture corn stover and perennial grasses (*63*, *65*, *67*). Losses can exceed this value due to damage to the plastic bag and difficulty collecting the material from the bag if it is not located on a well-drained surface. Further losses can occur during feed out if the removal rate is low (< 15 cm/day) or if densities are low, allowing air to diffuse into the bag. This phenomenon may be less of a concern for biomass stored in anaerobic structures because removal rates will be much higher for biomass compared to typical animal feeding operations due to differences in daily supply needs.

The exposed surfaces of bunkers and piles must also be sealed to prevent oxygen and moisture penetration. Here, too, polyethene is the plastic of choice. Sheets of this plastic are used to completely cover the crop with care to ensure overlapping when multiple sheets are needed to cover the whole pile.

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Table 2. Approximate densities of dry biomass forms

Package	*Density* *(kg DM)/)m^3*
Stack[1]	80
Bale	
Large Square[2]	150[3] - 200[2]
Round[3]	110[2]-1804
Chopped	
Loose[4]	70-80[2]
Packed[4]	150-160[2]

[1] ref (*56*). [2] refs (*27*, *63*). [3] refs (*27*, *64*). [4] refs (*63*, *65*).

Additionally, it is recommended that plastic be used along concrete and earthen walls to prevent moisture and oxygen that may have diffused through the wall from entering the pile. Plastic is commonly secured to the pile by rock bags or tire sidewalls. Keeping the plastic tight to the surface of the pile is an important management consideration that left unattended may lead to significant dry matter losses. Dry matter losses for these structures have been measured in excess of 10%, depending on management (*69*, *72*, *73*).

Fermentation acids (lactic, acetic, propionic and butyric) and ethanol vary depending on crop species, stage of maturity, growing conditions, moisture at harvest and microorganisms present. During typical fermentation conditions, the organic acids present in the largest quantities include lactic and acetic acid produced by hetero- and homo- fermentative lactic acid bacteria (LAB), respectively. Lactic acids typically range from 3 to 10 g/(kg DM). Lactate levels are typically higher for cool season grasses and for higher storage moistures and lower for drier forages as well as legumes and warm season grasses (*74*). Acetic acid follows a similar pattern, but values are observed at much lower levels and are typically between 0.5 and 3 g/(kg DM). In cases where heterofermentive bacteria (e.g., *Lactobacillus buchneri*) are dominate, acetate levels can approach 5 g/(kg DM). Biomass field research has shown that these acids are minimized with lower moisture contents while still producing an anaerobically stable product (*27*, *35*, *65*).

The final type of wet storage is film-wrapped round and large square bale forms. In this system, bales are stretch wrapped with a 25 μm low-density polyethene. The wrapping process stretches the film by about 25%, and approximately six to eight layers are applied for high and low moisture bales, respectively. The moistures for this system are quite a bit lower (40 to 55% w.b.), so the benefits of quick field wilting time are less compared to previously described storage systems, but they are still favorably shorter than dry storage. Additionally, the level of fermentation is restricted so fermentation acids are at lower levels (*35*, *65*). Finally, dry matter losses are similar to that of typical wetter ensiling systems.

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One disadvantage of this system over chopped materials is the additional material handling associated with bales. Also, film requirements can be high for individually-wrapped bales, but employing an in-line wrapper can reduce plastic use by up to 43% (*35*). Wrapping can be integrated into the baler to reduce bale handling, but the bales must be handled carefully as to not damage the film.

A final characteristic of the wet storage system is the possibility of chemical or biological pretreatment. For many bioconversion processes, biomass needs to be pretreated prior to further processing because the carbohydrates are largely contained in complex cell wall structures that impede their enzymatic conversion into fermentable sugars. Biomass recalcitrance also limits digestion in ruminants and, as such, there is a long history of chemically treating forages for increasing their energy values; only half of the cell wall carbohydrates are actually utilized when ruminants are fed untreated forage (*75*). In fact, many of the same pretreatments that have been investigated for biofuel applications have been previously evaluated on forages (e.g., lime and AFEX) (*76*).

More recently, it has been proposed that an on-farm ensilage process could promote storage and pretreatment of corn stover and perennial grass biomass that is amenable for fuel and fiber production (*77*). Addition of acids, alkalis, enzymes and ozone has been investigated (*78*, *79*). On-farm pretreatment has been shown to disrupt the cellulose-hemicelluloses-lignin matrix by release of acetyl groups from hemicellulose constituents and through improved bioconversion of cellulose to ethanol (*80*). Additionally, suppression of lactate may be attributed to the inhibition of lactic acid bacteria, demonstrating the preservative effect of the pretreatment systems.

Future Trends

Today, biomass crops are typically harvested using traditional forest, hay and forage harvesting equipment. In the future, biomass harvesting systems will evolve as the market demands more specialized machines. Specifically, harvesting systems will need to be developed to reduce the number of field operations required to harvest biomass, improving the energy balance and economics. Additionally, future harvesting machines will need to be more robust to withstand the rigors of harvesting high-yielding crops and high annual usage. New harvesting systems will employ a high level of technology for data acquisition and management. Machine data will be enhanced through on-harvester implementation of existing and novel sensor technologies to spatially assess the physical and chemical properties of harvested material. Telecomunications systems will be used to provide this information in real-time to manage feedstock logistics systems as well as to document energy, mass and compositional flows through feedstock storage, processing and distribution networks. Biomass harvesting machines will require large capital investments and demand sophisticated management skills, so these machines will increasingly be owned and operated by businesses specializing in biomass logistics.

In the future, additives or amendments could be applied to biomass to preserve or change the chemical properties of the feedstock in storage. Densification processes could be applied to the feedstocks prior to transport to reduce shipping

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costs. Biomass may also be processed in regional processing facilities to improve transport properties and enhance the value of the feedstock before end-use.

References

1. Hartsough, B.; Yomogida, D. *Compilation of State-of-the-Art Mechanization Technologies for Short-Rotation Woody Crop Production*; Department of Biological and Agricultural Engineering Department, University of California-Davis: Davis, CA,1996; 66 p.
2. Mitchell, C. P.; Stevens, E. A.; Watters, M. P. Short-rotation forestry – Operations, productivity and costs based on experience gained in the UK. *For. Ecol. Manage.* **1999**, *121*, 123–136.
3. Deboys, R. *Harvesting and Comminution of Short-Rotation Coppice*; ETSU contractor report, (Technical Development Branch, FC), No. ETSU B/W2/ 00262/REP;1996.
4. Lechasseur, G.; Savoie, P. *Cutting, Bundling and Chipping Short Rotation Willow*; CSAE/SCGR Paper 05-080; 2005.
5. Buchholz, T.; Volk, T. 2010.
6. Dawson, M. *Short Rotation Coppice Willow – Best Practice Guidelines*, 2007. Renew Project Omagh College. http://www.ruralgeneration.com/BEST%20PRACTICE%20GUIDE.pdf (accessed September, 2010).
7. Persson, S. *Mechanics of Cutting Plant Material*; American Society of Agricultural and Biological Engineers (ASABE): St. Joseph, MI, 1987.
8. Labrecque, M.; Teodorescu, T. High biomass achieved by Salix clones in SRIC following two 3-year coppice rotations on abandoned farmland in southern Quebec, Canada. *Biomass Bioenergy* **2003**, *25* (2), 135–146.
9. Idler, C.; Scholz, V.; Daries, W.; Egert, J. *Loss Reduced Storage of Short Rotation Coppice*; Zemes Ukio Inzinerija: Mokslo Darbai, 2005; Vol. 37, Issue 1, pp 124−134.
10. Stucley, C. R.; Schuck, S. M.; Sims, R. E. H.; Larsen, P. L.; Turvey, N. D.; Marino, B. E. *Biomass Energy Production in Australia*; Rural Industries Research and Development Corporation Publication No. 04/031, RIRDC Project No EPL-1A; 2008.
11. White, M. S.; DeLuca, P. A. Bulk storage effects on the fuel potential of sawmill residues. *For. Prod. J.* **1978**, *28* (11), 24–29.
12. Kubler, H. Air convection in self-heating piles of wood chips. *Tappi J.* **1982**, *65* (8), 63–79.
13. White, M. S.; Curtis, M. L.; Sarles, R. L.; Green, D. W. Effects of outside storage on the energy potential of hardwood particulate fuels: Part 1. Moisture content and temperature. *For. Prod. J.* **1983**, *33* (6), 31–38.
14. Fuller, W. S. Chip pile storage: A review of practices to avoid deterioration and economic losses. *Tappi J.* **1985**, *68* (8), 48–51.
15. Jirjis, R. Effects of particle size and pile height on storage and fuel quality of comminuted *Salix viminalis*. *Biomass Bioenergy* **2005**, *28* (2), 193–201.
16. Kubler, H. Heat generation processes as cause of spontaneous ignition in forest products. *For. Prod. Abstr.* **1987**, *10* (11), 299–322.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch003

17. Hartmann, H.; Bohm, T.; Jensen, P. D.; Temmerman, M.; Rabier, F.; Golser, M. Methods for size classification of wood chips. *Biomass Bioenergy* **2006**, *30*, 944–953.
18. Jensen, P. D.; Mattsson, J. E.; Kofman, P. D.; Klausner, A. Tendency of wood fuels from whole trees, logging residues and roundwood to bridge over openings. *Biomass Bioenergy* **2004**, *26*, 107–113.
19. SCAN-CM 40:01, *Wood Chips for Pulp Production—Size Distribution*. Scandinavian Pulp, Paper and Board Testing Committee, 2001. http://www.nordstand.com/upload/NSP/SCAN-test%20Methods/Series%20C/CM%2040-01.pdf.
20. Lewis, D. *The Use of Wood Fuel for Energy Generation: Opportunity or Just Hot Air?* RICS Research Conference - ROOTS 1999. ISBN 0-85406-945-3.
21. Shinners, K. J.; Binversie, B. N.; Savoie, P. *Whole-Plant Corn Harvesting for Biomass: Comparison of Single-Pass and Multi-Pass Harvest Systems*; ASABE Paper No. 036089; ASABE: St. Joseph, MI, 2003.
22. Lavoie, F.; Savoie, P.; D'Amours, L.; Joannis, H. Development and field performance of a willow cutter-shredder-baler. *Appl. Eng. Agric.* **2008**, *24* (2), 165–172.
23. Wright, N. A. Screening of Herbaceous Species for Energy Crops on Wet Soils in Ohio. In *Advances in New Crops*; Janick, J., Simon, J. E., Eds.; Timber Press: Portland, OR, 1990; pp 263−267.
24. Thomason, W. E.; Raun, W. R.; Johnson, G. V.; Taliaferro, C. M.; Freeman, K. W.; Wynn, K. J.; Mullen, R. W. Switchgrass response to harvest frequency and time and rate of applied nitrogen. *J. Plant Nutr.* **2004**, *27* (7), 1199–1226.
25. Parrish, D. J.; Fike, F. H. The biology and agronomy of switchgrass for biofuels. *Plant Sci.* **2005**, *24* (5-6), 423–459.
26. Geber, U. Cutting frequency and stubble height of reed canarygrass (*Phalaris arundinacea* L.): Influence on quality and quantity of biomass for biogas production. *Grass Forage Sci.* **2002**, *57*, 389–394.
27. Shinners, K. J.; Boettcher, G. C.; Muck, R. E.; Weimer, P. J.; Casler, M. D. Harvest and storage of two perennial grasses as biomass feedstocks. *Trans. ASABE* **2010a**, *53* (2), 359–370.
28. Lewandowski, I.; Scurlock, J. M. O.; Lindvall, E.; Christou, M. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass Bioenergy* **2003**, *25*, 335–361.
29. Alder, P. R.; Sanderson, M. A.; Boateng, A. A.; Weimer, P. J.; Jung, H. G. Biomass yield and biofuel quality of switchgrass harvested in fall or spring. *Agron. J.* **2006**, *98*, 1518–1525.
30. Rotz, C. A.; Chen, Y. Alfalfa drying model for the field environment. *Trans. ASABE* **1985**, *28* (5), 1686–1691.
31. Bransby, D. I.; Sladden, S. E.*Yield Effects on Bale Density and Time Required for Commercial Harvesting and Baling of Switchgrass*. Proceedings of Bioenergy '96, Nashville, TN, September 15−20, 1996.
32. Kristensen, E. F.*Harvesting and Handling of Miscanthus – Danish Experince*, Proceeding of 1st Meeting of IAE – Bioenergy Task 30. In DIAS

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch003

Report – Plant Production; Jorgensen, U., Virwijst, T., Eds., 2003, Vol. 86, pp 41−46.

33. USDA Feed Grain Baseline, 2009-18. U.S. Department of Agriculture: Washington, DC, 2009. http://www.ers.usda.gov/Briefing/Corn/2009baseline.htm (accessed August, 2010).
34. Perlack, R. D.; Wright, L. L.; Turnhollow, A. F.; Graham, R. L.; Stokes, B. J.; Erbach, D. C. *Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-Ton Annual Supply*; ORNL/TM-2005/66; U. S. Department of Energy and U. S. Department of Agriculture, Forest Service, 2005, 73 p.
35. Shinners, K. J.; Huenink, B. M.; Muck, R. E.; Albrecht, K. A. Storage characteristics of large round and square alfalfa bales: Low-moisture wrapped bales. *Trans. ASABE* **2009b**, *52* (2), 401–407.
36. Albert, W. W. ; Stephens, L. E. *Stalklage Silage Harvested with a Converted Combine*; ASAE Paper No. 69-313; ASABE: St. Joseph, MI, 1969.
37. Ayres, G. E.; Buchele, W. F. *An Evaluation of Machinery Systems for Harvesting Corn Plant Forage*; ASAE Paper No. 76-1015; ASABE: St. Joseph, MI, 1976.
38. Hitzhusen, T. E.; Marley, S. J.; Buchele, W. F. Beefmaker II: Developing a total corn harvester. *Agric. Eng.* **1970**, *51*, 632–634.
39. Shinners, K. J.; Adsit, G. S.; Binversie, B. N.; Digman, M. F.; Muck, R. E.; Weimer, P. J. Single-pass, split-stream of corn grain and stover. *Trans. ASABE* **2007a**, *50* (2), 355–363.
40. Shinners, K. J.; Bennett, R. G.; Hoffman, D. S. *Single- and Two-Pass Corn Stover Harvesting Systems*; ASABE Paper No. 095652; 2009c.
41. Kass, K. J. Apparatus and Method for Harvesting and Windrowing Corn. U.S. Patent Number 4,182,098, 1980.
42. McLeod, R. H. Mobile Harvester for Chaff, Grain, Grain Leavings and Weed Seeds. U.S. Patent Number 5,794,423, 1998.
43. Yore, M. W.; Summers, M. D.; Jenkins, B. M. *Development of a Stubble Cutting System for a Combine Harvester*; ASABE Technical Paper 011087; ASABE: St. Joseph, MI, 2001.
44. Siemens, M. C.; Hulick, D. E. A new grain harvesting system for single-pass grain harvest, biomass collection, crop residue sizing and grain segregation. *Trans. ASABE* **2008**, *51* (5), 1519–1527.
45. Skaar, C. *Wood Water Relations*; Springer-Verlag: NewYork, 1988; 283 p.
46. Nystrom, J.; Dahlquist, E. Methods for determination of moisture content in woodchips for power plants – A review. *Fuel* **2004**, *83*, 773–779.
47. Van loo, S.; Koppejan, J. *Handbook of Biomass Combustion and Co-Firing*; Twente University Press: Enschede, Netherlands, 2002, ISBN 9036517737.
48. Jirjis, R. Storage and drying of wood fuel. *Biomass Bioenergy* **1990**, *9*, 181–190.
49. Richardson J.; Bjorheden R.; Hakkila P.; Lowe A. T.; Smith C. T. *Bioenergy from Sustainable Forestry: Guiding Principles and Practice*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2002; 344 p.
50. Kollmann, F. F. P.; Cote, W. A. J. Principles of Wood Science and Technology. In *I. Solid Wood*; Springer-Verlag: NewYork, 1968; 592 p.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch003

51. Jirjis, R; Theander, O. The effect of seasonal storage on the chemical composition of forest residue chips. *Scand. J. For. Res.* **1990**, *5*, 437–448.
52. Mani, S.; Tabil, L. G.; Sokhansanj, S. Effects of compressive force, particle size and moisture content on mechanical properties of biomass pellets from grasses. *Biomass Bioenergy* **2006**, *30*, 648–654.
53. Li, Y.; Liu, H. High pressure densification of wood residues to form an upgraded fuel. *Biomass Bioenergy* **2000**, *19*, 177–186.
54. Kaliyan, N.; Morey, R. V. Factors affecting strength and durability of densified biomass products. *Biomass Bioenergy* **2009**, *33*, 337–359.
55. Chin, O. C.; Siddiqui, K. M. Characteristics of some biomass briquettes prepared under modest die pressures. *Biomass Bioenergy* **2000**, *18*, 223–228.
56. Sokhansanj, S.; Mani, S.; Turhollow, A.; Kumar, A.; Bransby, D.; Lynd, L.; Laser, M. Large-scale production, harvest and logistics of switchgrass - Current technology and envisioning a mature technology. *Biofuels, Bioprod. Biorefin.* **2009**, *3* (2), 124–141.
57. Demirbas, A. Effects of moisture and hydrogen content on the heating value of fuels. *Energy Sources Part A* **2007**, *29*, 649–655.
58. Hartley, I. D.; Wood, L. J. Hygroscopic properties of densified softwood pellets. *Biomass Bioenergy* **2008**, *32*, 90–93.
59. Lehtikangas, P. Storage effects on pelletised sawdust, logging residues and bark. *Biomass Bioenergy* **2000**, *19*, 287–293.
60. Kuokkanen, M.; et al. Chemical methods in the development of eco-efficient wood-based pellet production and technology. *Waste Manage. Res.* **2009**, *27*, 561–571.
61. Svedberg, U.; Hogberg, H.; Hogberg, J. Emission of hexanal and carbon monoxide from storage of wood pellets, a potential occupational and domestic health hazard. *Br. Occup. Hyg. Soc.* **2003**, *48* (4), 339–349.
62. Curci, M. J. Procurement, Process, and Storage Techniques for Controlling Off-Gassing and Pellet Temperatures, 2010. http://pelletheat.org/wp-content/uploads/2010/07/Pellet-off-gassing-study.pdf (accessed September, 2010).
63. Shinners, K. J.; Binversie, B. N.; Muck, R. E.; Weimer, P. J. Comparison of wet and dry corn stover harvest and storage. *Biomass Bioenergy* **2007b**, *31* (4), 211–221.
64. Shinners, K. J.; Huenink, B. M.; Muck, R. E.; Albrecht, K. A. Storage characteristics of large round alfalfa bales: Dry hay. *Trans. ASABE* **2009a**, *52* (2), 409–418.
65. Shinners, K. J.; Wepner, A. D.; Muck, R. E.; Weimer, P. J. Aerobic and anaerobic storage of single-pass, chopped corn stover. *BioEnergy Res.* **2010b**, DOI: 10.1007/s12155-010-9101-7.
66. Harrigan, T. M.; Rotz, C. A. Net, plastic and twine-wrapped large round bale storage losses. *Appl. Eng. Agric.* **1994**, *10* (2), 189–194.
67. Huhnke, R. L. Large round bale alfalfa hay storage. *Appl. Eng. Agric.* **1988**, *4* (4), 316–318.
68. Wiselogel, A. E.; Agblevor, F. A.; Johnson, D. K.; Deutch, S.; Fennell, J. A.; Sanderson, M. A. Compositional changes during storage of large round switchgrass bales. *Bioresour. Technol.* **1996**, *56* (1), 103–109.

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69. McDonald, P. *The Biochemistry of Silage*; John Wiley and Sons, Inc.: New York,1981.
70. Muck, R. E.; Holmes, B. J. Factors affecting bunker silo densities. *Appl. Eng. Agric.* **2000**, *16* (6), 613–619.
71. Muck, R. E.; Holmes, B. J. Bag silo densities and losses. *Trans. ASABE* **2006**, *49* (5), 1277–1284.
72. Buckmaster, D. R.; Rotz, C. A.; Muck, R. E. A comprehensive model of forage changes in the silo. *Trans. ASABE* **1989**, *32* (4), 1143–1152.
73. Ruppel, K. A.; Pitt, R. E.; Chase, L. E.; Galton, D. M. Bunker silo management and its relationship to forage preservation on dairy farms. *J. Dairy Sci.* **1995**, *78* (1), 141–153.
74. Kung, L.; Shaver, R. Interpretation and Use of Silage Fermentation Analysis Reports. http://www.dairylandlabs.com/pages/interpretations/vfa.php (accessed August 2010).
75. Hatfield, R. D.; Ralph, J.; Grabber, J. H. Cell wall structural foundations: Molecular basis for improving forage digestibilities. *Crop Sci.* **1999**, *39* (1), 27–37.
76. Sundstol, F.; Owen, E. *Straw and Other Fiberous By-Products as Feed*; Elsevier Science Publishers B.V.: Amsterdam, The Netherlands, 1984.
77. Richard, T. L.; Proulx, S.; Moore, K. J.; Shouse, S. *Ensilage Technology for Biomass Pre-Treatment and Storage*; ASABE Paper No. 016019; ASABE: St. Joseph, MI, 2001.
78. Digman, M. F.; Shinners, K. J.; Dien, B. S.; Muck, R. E.; Xin-Liang, L.; Hatfield, R. D.; Weimer, P. J. *On-Farm Pretreatment Technologies for Improving Enzymatic Degradability of Cellulose and Hemicellulose Present in Perennial Grass*; ASABE Paper No. 071021; ASABE: St. Joseph, MI, 2007.
79. Ren, H. Y.; Richard, T. L.; Moore, K. J. The impact of enzyme characteristics on corn stover fiber degradation and acid production during ensiled storage. *Appl. Biochem. Biotechnol.* **2007**, *137*, 221–238.
80. Digman, M. F.; Shinners, K. J.; Casler, M. D.; Dien, B. S.; Hatfield, R. D.; Jung, H.-J. G.; Muck, R. E.; Weimer, P. J. Optimizing on-farm pretreatment of perennial grasses for fuel ethanol production. *Bioresour. Technol.* **2010**, *101* (14), 5305–5314.

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Chapter 4

Physical Pretreatment − Woody Biomass Size Reduction − for Forest Biorefinery

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Physical pretreatment of woody biomass or wood size reduction is a prerequisite step for further chemical or biochemical processing in forest biorefinery. However, wood size reduction is very energy intensive which differentiates woody biomass from herbaceous biomass for biorefinery. This chapter discusses several critical issues related to wood size reduction: (1) factors affecting mechanical energy consumption and the post-pretreatment wood size-reduction approach to significantly reduce energy consumption, (2) biomass substrate specific surface area for substrate size characterization and the wet imaging technique for woody substrate size/specific surface measurements, (3) the effect of biomass substrate size/specific surface on enzymatic cellulose hydrolysis, and (4) the concept of substrate "surface productivity" for the determination of optimal degree of size reduction and energy efficient wood size reduction.

Keywords: woody/forest biomass; forest biorefinery; size reduction; enzymatic hydrolysis/saccharification; size characterization

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Introduction

Woody biomass represents a significant portion of biomass that can be sustainably produced in the United States (*1*) and around the world. Woody biomass has several advantages for the future biobased economy, such as high density that reduces transportation cost and flexible harvesting time that eliminates long-term storage, and low ash content that reduces processing dead load (*2*). To promote biodiversity and meet local and regional bioenergy needs, woody materials will be a critical part of the biomass supply mix in the future biobased economy.

Effective separation and fractionation of different components of woody biomass are critical to forest biorefinery. Woody biomass consists of various components, each of which can be used to produce a variety of bioproducts, including biofuel. For example, cellulose has been traditionally used to produce fiber to make pulp and paper through delignification. Cellulose can also be saccharified by enzymes to produce sugars to be converted to biofuel. However, wood is a structural material, physically large and structurally tough, and has natural resistance to microbial destruction. To improve wood cellulose accessibility to enzymes for biochemical conversion to sugars, physical and chemical barriers need to be removed. This is often achieved by pretreatment, a preprocessing step using mechanical, thermal, and thermo-chemical means (*2*). Physical pretreatment; i.e., wood size reduction through mechanical means, can increase the accessible surface area of wood cellulose to enzymes. This allows effective separation/fractionation for efficient utilization of different wood components through biorefining by chemical, thermal, and/or microbial processing (*3*–*5*).

Feedstock pretreatments reported in the literature have almost exclusively focused on chemical pretreatments. Physical pretreatment; i.e., biomass size reduction, has been largely overlooked. This is partly because most reported studies were focused on herbaceous biomass that does not need significant processing and energy to achieve desired size reduction (*6*). Such preprocessing of herbaceous biomass can be achieved during harvesting using combines. Whereas a certain degree of size reduction of woody biomass is possible during harvesting using modern technologies designed specifically for harvesting woody energy crops, it can only achieve the first step in size reduction (*2*, *4*), i.e., from trees or logs to wood chips. Size reduction of woody biomass is very similar to wood fiber production, which involves two steps (*2*, *4*). The first step is chipping logs or branches to produce wood chips with a size around 10–50 mm in two dimensions and 5–15 mm in the third dimension. The second step is fiberization or pulverization of wood chips to produce wood fibers/fiber bundles or flour. Wood chipping is a common practice in the pulp and paper industry, and energy costs are relatively low compared with those for fiberization or pulverization (*2*). Therefore, this chapter is dedicated to the second step, wood-size reduction, that directly affects the end products through subsequent processing: separation and fractionation.

Several well developed technologies are available for biomass size reduction, such as hammer milling, knife milling, shredding, and disk or attrition milling.

Several studies reported biomass size reduction using these technologies (*6*–*9*). Early work on size reduction of woody biomass included those producing wood flour for manufacturing wood composites (*10*). Hammer and disk milling are the two predominant technologies from a large-scale production point of view. Hammer milling has been mainly used for producing wood flours for composites and pellets, whereas disk milling has been used for wood fiber production. Disk milling represents one of the best fiberization processes for woody feedstock in commercial fiber production at the scale of 1,000 tons/day, which is equivalent to an ethanol production capacity of 100 million L/year. The fiberization process of disk milling is favorable for efficient enzymatic saccharification (*4*). Therefore, we will focus on disk milling in the following discussion.

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Energy Consumption in Wood Size Reduction

Wood-size size reduction is energy intensive, which makes physical pretreatment of woody biomass particularly important. For example, typical electrical–mechanical energy consumption for disk milling wood chips under atmospheric conditions to the level of fibers/fiber bundles is about 150–800 kWh (or 0.54–2.88 GJ)/ton wood (*3*, *8*). Energy consumption to produce wood chips is about 50 kWh (0.18 GJ)/ton wood. The total thermal energy of ethanol produced from wood is estimated at 7.2 GJ/ton wood based on an ethanol yield of 300 L/ton of od wood with current technology and a higher heating value of ethanol of about 24 MJ/L. Therefore, size-reduction energy consumption is about 10 to 40% of the thermal energy of ethanol produced from wood. Considering the conversion efficiency of 30% from thermal energy to electric–mechanical energy consumed in wood-size reduction, the thermal energy in ethanol produced from wood is just sufficient for wood-size reduction. Because energy is the major product of a biorefinery operation, one has to achieve good net energy output in addition to profitability, or it will be necessary to seek energy elsewhere. Therefore, significant reduction in energy consumption for wood-size reduction, preferably by a factor of 5–10 to about 0.1–0.4 GJ/ton, is required to achieve sensible net energy output from wood ethanol production. This requirement poses a significant challenge to biofuel production from woody biomass.

Several factors affect energy consumption for wood-size reduction through disk milling (*2*). The first and foremost factor is the fiberization mechanism. It is well understood that energy consumption in mechanical wood pulping depends significantly on how the wood chips are fiberized (*2*). Depending on the disk-milling conditions, wood chips can be fractured through the lumen of wood tracheids, such as in producing refiner mechanical pulps (RMP) under atmospheric conditions. Wood chips can also be fractured in the S1 and S2 layer of cell wall when wood chips are steam pretreated, such as in producing thermomechanical pulps (TMP) in which low-pressure steam of about 2-4 bar (~120-140°C) is used to soften wood chips before disk refining. When the steam pressure is increased to 7.2 bar, as the steam temperature (~166°C) exceeds the glass transition temperature of lignin (*11*), wood chips are fractured in the lignin-rich middle lamella (ML). This high-pressure treatment of wood chips is used to produce medium-density fiberboard pulps (MDF). Figure 1 shows the schematic diagram of different wood chip fiberization mechanisms. The mechanical energy consumption varies significantly among different fiberization mechanisms.

Typical energy consumption in the first pass refining of wood chips for producing RMP, TMP, and MDF are about 800, 500, and 150 kWh/ton oven-dry (od) wood based on numerous laboratory experiments and pulp mill commercial practice (*2*). The energy consumption in first pass refining of chemically pretreated wood chips (CTMP) is often lower than that for TMP. The surface chemical compositions of the pulp fibers produced by different fiberization mechanisms are very different. RMP exposes mostly cellulose on fiber surfaces, whereas MDF fibers are coated with lignin on their surface. This can be clearly seen from the color of these pulps with RMP being the lightest and MDF being browner and darker. The difference in surface chemical composition certainly affects cellulose enzymatic saccharification, as revealed in our previous study (*4*).

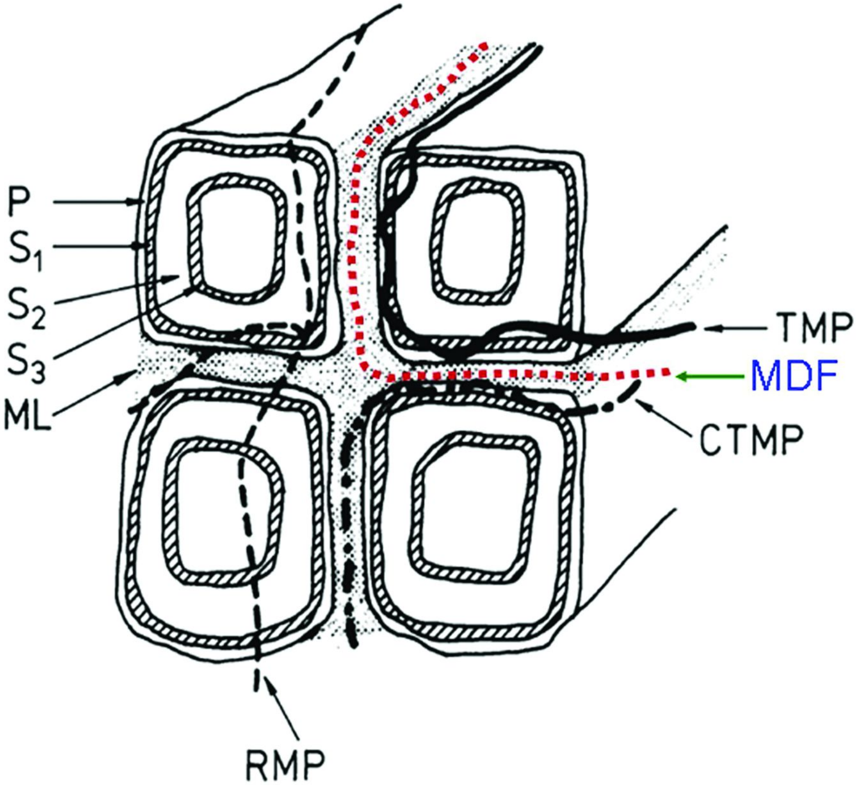

*Figure 1. A schematic diagram showing the various fiberization mechanisms of softwood. Adapted with permission from Franzen, Nordic Pulp Paper Res. J., **1**:4, 1986 and Salmen, Fundamentals of Mechanical Pulping, in Book 5: Mechanical Pulping, Papermaking Science and Technology, Gullichsen and Paulapuro Eds., Fapet Oy, Finland, 1999. Copyrights 1986 Nordic Pulp and Paper Research Journal and 1999 Finland Paper Engineers' Association.*

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The second factor affecting energy consumption for wood size reduction is the degree of size reduction. Generally speaking, more energy is required to produce substrate with a large surface area (fine material). Characterization of biomass substrate is necessary to fully address this issue. Unfortunately, proper characterization of biomass substrate size is a difficult task, as will be discussed later.

Disk milling conditions certainly affect energy consumption for wood size reduction. Conditions can alter wood chip fiberization mechanism, as discussed previously. They can also affect the substrate morphology and size and thereby affect energy consumption without affecting fiberization mechanism. For example, opening the disk-plate gap in refining results in low energy consumption, and a coarse substrate that may contain fiber bundles (*3*). One study demonstrated that in mechanical pulping, the disk-refining (milling) process conditions can significantly affect disk-refining energy consumption (*12*). However, the energy savings that can be realized without sacrificing pulp quality is very limited in mechanical pulping (*13*). For example, decreasing primary-stage disk-refining discharge consistency (the same as solids-loading) from 50 to 38% only resulted in 7% energy savings in commercial-scale trial runs (*14*). However, the fiber qualities required for papermaking are not an issue for woody biomass saccharification. This makes it feasible to optimize milling process conditions to significantly decrease energy consumption for wood-size reduction while maximizing the enzymatic cellulose saccharification efficiency under nominal chemical pretreatment conditions.

Our recent study using lodgepole pine found that reducing solids-loading in disk milling from 30% (the solids of the wood chips right after pretreatment) to about 20% can reduce disk-milling energy by 20% (*3*). We also found that opening the disk-plate gap from 0.38 mm to 1.52 mm can reduce disk milling energy by 75% (or a factor of 4) when wood chips were pretreated by dilute acid or the Sulfite Pretreatment to Overcome Recalcitrance of Lignocelluloses (SPORL) process (*3*). No negative effects on the enzymatic saccharification of the resultant substrates were observed in the ranges of disk-milling solids loading and disk plate gaps (0.38-1.52 mm) studied (*3*). Near complete saccharification was achieved for lodgepole pine pretreated by SPORL followed by disk milling with energy consumptions of 20–50 kWh/ (0.07-0.18 GJ/ton).

Post-Pretreatment Wood-Size Reduction

The approach of biomass size reduction prior to chemical pretreatment has been proposed as the standard process flow for biofuel production by several key pieces of the literature (*15*, *16*) and by the recent U.S. Department of Energy biofuels research roadmap (*17*). This approach has a significant negative effect on the overall energy balance and net energy output in biofuel production from woody biomass because wood-size reduction is very energy intensive. As discussed in the previous section, thermal or chemical pretreatment of wood chips can alter the wood chip fiberization mechanism in disk milling and thereby reduce wood chip size-reduction energy consumption (comparing RMP, TMP, and CTMP). Furthermore, these pretreatments can also alter the chemical composition and physical structure of wood by partially removing and modifying some cell-wall components such as hemicellulose and lignin, which can lead to reduced energy consumption for milling the pretreated wood. We therefore have proposed to conduct size reduction after chemical pretreatment, i.e., post-chemical pretreatment size reduction, as shown in Figure 2, to significantly reduce the energy consumption for wood-size reduction (*2*, *3*, *5*, *18*).

This approach is also used in steam explosion pretreatment through the post-steam-pretreatment discharge (flashing or explosion) process. Energy savings of about 30% were obtained in mechanical pulping of papermaking fibers when wood chips were pretreated by oxalic acid to partially remove wood hemicelluloses (*19*). Table I lists energy savings in mechanical milling of lodgepole pine wood chips pretreated by different chemical pretreatments. A factor of 4 savings in size-reduction energy consumption was achieved when a SPORL process (*18*, *20*) was applied to lodgepole pine wood chips. Furthermore, near complete enzymatic saccharification was achieved for the substrates from disk-milling lodgepole (softwood) wood chips pretreated by the SPORL process. The energy consumption for the disk milling of a SPORL (pH = 1.9) pretreated wood chips reported in Table I and our previous work are equivalent to that used for size reduction by steam explosion. The thermal energy used for size-reduction through flashing by steam explosion can be estimated to approximately 0.4 GJ/ton wood by using the difference in enthalpy between saturated steam and saturated water with consideration of thermal enery recovery in the form of low pressure steam and hot water, respectively. It should be pointed out that the total energy consumption for conducting steam explosion at 215ºC is estimated at 1.8 GJ/ton wood (*2*) and used for both thermal pretreatment and size redudction.

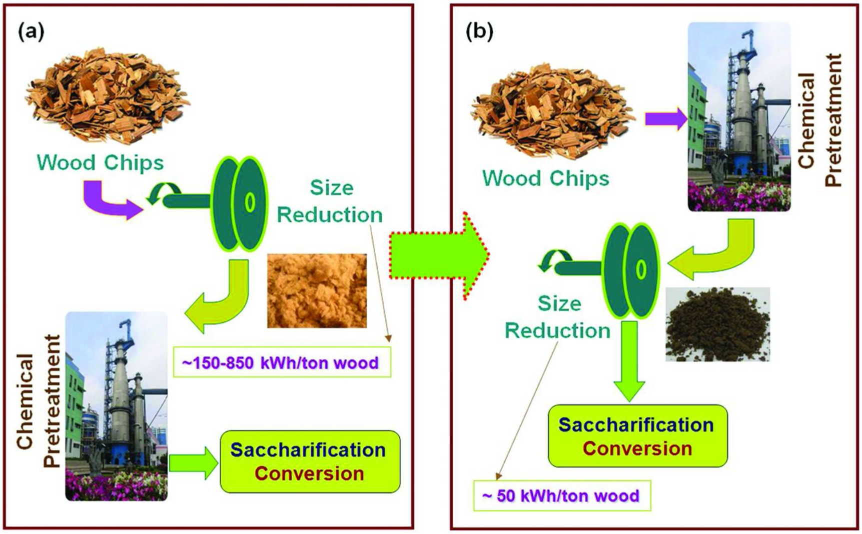

Figure 2. Schematic diagram shows the approach for mechanical wood size-reduction operation from (a) pre- to (b) post-chemical pretreatment to reduce energy consumption. Reproduced from reference (5).

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Table I. Effects of chemical pretreatment on lodgepole pine wood chip size-reduction energy consumption and the resultant substrate enzymatic digestibility (SED). Pretreatment liquor to wood rato (L/W) = 3, disk milling solids loading = 30% (≈ the solid contents of pretreated wood chips), and disk plate gap = 0.76 mm. Reproduced from reference (*5*)

Pretreatment @180°C for 30 min[a]	*Initial liquor pH*	*Disk milling energy (kWh/ton wood)*	*Size-reduction energy savings (%)*	*SED (%)*
Untreated		699		12.7
Hot-water	5.0	680	2.7	16.0
Acid	1.1	412	41.0	41.6
SPORL	4.2	594	15.0	75.1
SPORL	1.9	153	78.1	91.6

[a] Sodium bisulfite charge was 8% on oven dry (od) wood for the two SPORL runs; sulfuric acid charge was 2.21 (w/w) on od wood for the dilute acid and low pH SPORL runs, and 0 for the hot-water and high pH SPORL runs.

The post-chemical pretreatment size-reduction approach has several benefits: (1) it significantly reduces mechanical energy consumption for wood-size reduction by taking advantage of chemical pretreatments to remove solid wood mass and soften wood structure; (2) it avoids the difficult and energy-intensive operation of mixing high-consistency pulp (size-reduced/fiberized woody material) with chemicals during pretreatment; (3) it also avoids the difficult solid (fibers) and liquid (pretreatment hydrolysate) separation process after pretreatment; and (4) it affords a low liquid to woody biomass ratio in thermo-chemical pretreatments, which reduces thermal energy consumption and potentially produces a concentrated hemicellulose sugar stream favorable for fermentation and reducing downstream separation and distillation cost; the liquid uptake of wood chips is much lower than fibers and/or fiber bundles because of the porous and hydrophilic nature of wood fibers.

Substrate Size Characterization

Proper characterization of substrate size is important to determine the degree of wood size reduction that affects not only energy consumption but also enzyme accessibility and therefore substrate enzymatic digestibility. The particle-size distribution of a given substrate can be measured by a variety of techniques. The traditional techniques are the sieve and screen methods. Modern techniques include imaging analysis. Imaging techniques often measure the projection dimensions of a particle. The geometric mean diameter of the particles has commonly been used to characterize biomass substrate (*9*). This is probably because size measurements were often carried out by traditional sieve or screen methods (*6*, *9*). This size measure is significantly affected by biomass substrate morphology such as particle aspect ratio (*4*). Depending on the size-reduction method used, the same mesh size can mean completely different particles. For example, the fraction of hammer-milled spruce (HM-R80) passing a 48 mesh screen and being retained on an 80 mesh screen is much coarser (larger in size) than the fraction retained on a 48 mesh screen from the disk milling (DM-R48) of the same wood, as shown in Figure 3. Most size-reduction processes produce fibrous substrate with wide ranges of particle (fiber) aspect ratios over 10. As a result, existing data on substrate size characterization using sieve and screen methods have limited value.

Substrate surface area is most relevant to heat and mass transfer, enzyme accessibility, and energy consumption; therefore, the substrate-specific surface should be used to characterize the size of a substrate. Specific surface area has been used to correlate energy consumption for comparing the efficiencies of several size-reduction processes (*7*). The calculation of specific surfaces was based on the assumption that the substrate consists of spherical particles. Under this assumption, the physical dimension is often represented by its mean diameter. Several statistical ways can calculate the mean particle diameter. The arithmetic mean D_{10}, Sauter mean D_{32}, and volume (mass) mean D_{30} diameters can be calculated (*21*, *22*). Neglecting particle surface roughness, the external volumetric specific surface, S_p^V, can be estimated according to the equation

$$S_p^V = \frac{A_p}{V_p} = 6 \cdot \frac{\sum_i n_i d_i^2}{\sum_i n_i d_i^3} = \frac{6}{D_{32}} \qquad (1)$$

where A_p, and V_p are the total surface area and volume of the particles, respectively, and n_i is the number of particles in size bin i with representative diameter d_i. D_{32} is also called Sauter mean diameter (*SMD*). By measuring the oven dry weight of the sample, m_p, before analysis, the specific surface, S_P, of the sample can be determined using the following expression when each fiber (particle) in the sample is accounted for and measured:

$$S_p = \frac{A_p}{m_p} = \frac{\pi \sum_i n_i d_i^2}{m_p} \qquad (2)$$

The spherical model is not suitable for biomass substrate as biomass particles are not spheres but shives or spindles with very large aspect ratios (Figures 3 and 4). The substrates derived from disk-milling processes typically have an aspect ratio of 50 to 100. Therefore, measurements in more than one dimension are required to estimate substrate-specific surface by using non-spherical particle models. Furthermore, measurements need to be carried out under wet conditions, as enzymatic hydrolysis of lignocelluloses is always conducted in aqueous solutions. The wet-imaging technique is a technology that is commercially available and has been widely applied in pulp and paper science for fiber length characterization. When the spatial resolution of the image system is

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increased to resolve the width or diameter of a fiber, the system can be used for two-dimensional characterization of a lignocellulosic substrate. Assuming individual fibers are cylinders and neglecting surface roughness, the volumetric specific surface of fibers, S_p^V, can be estimated according to the following equation (*4*),

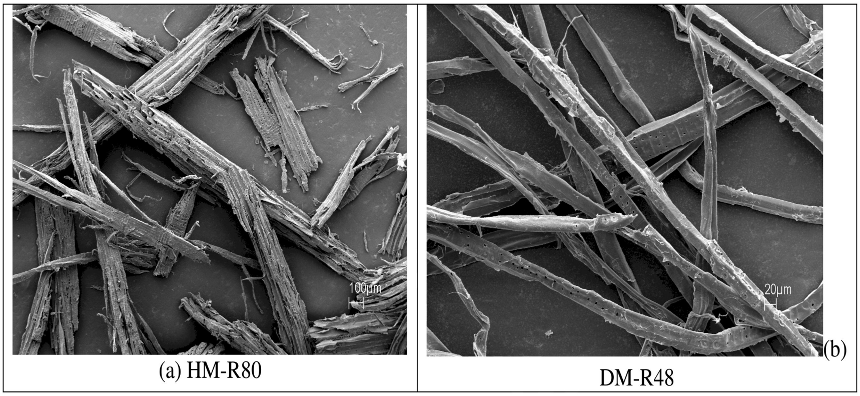
$$S_f^{\ V} = \frac{A_f}{V_f} = \frac{2\sum_i n_i(d_i^{\ 2} + 2\cdot d_i\cdot L_i)}{\sum_i n_i d_i^{\ 2}\cdot L_i} = \frac{4\sum_i n_i d_i(L_i + d_i/2)}{\sum_i n_i L_i\cdot d_i^{\ 2}} \qquad (3)$$

For most fibers with aspect ratios greater than 5, Eq. (3) can be approximated to

$$S_f^{\ V} \approx 4\cdot\frac{\sum_i n_i L_i\cdot d_i}{\sum_i n_i L_i\cdot d_i^2} = \frac{4}{D_{L21}} \qquad (4)$$

where A_f and V_f are the total surface area and volume of the fibers, respectively. n_i is the number of fibers in fiber group *i*. L_i and d_i are the representative length and diameter of fiber group *i*, respectively. D_{L21} is a fiber-length weighted-surface-length mean fiber diameter or "width." Similarly, the specific surface of the sample, S_f, can be determined by measuring the oven dry weight of the sample, m_f, before analysis when each fiber (particle) in the sample is accounted for and measured:

$$S_f = \frac{A_f}{m_f} = \frac{\pi}{2}\frac{\sum_i n_i(d_i^{\ 2} + 2\cdot d_i\cdot L_i)}{m_f} \approx \pi\frac{\sum_i n_i\cdot d_i\cdot L_i}{m_f} \qquad (5)$$



(a) HM-R80

(b) DM-R48

Figure 3. Comparison of the morphology of spruce substrates produced from hammer milling (HM) and disk milling (DM), (a) HM-R80, (b) DM-R48. Reproduced from reference (4).

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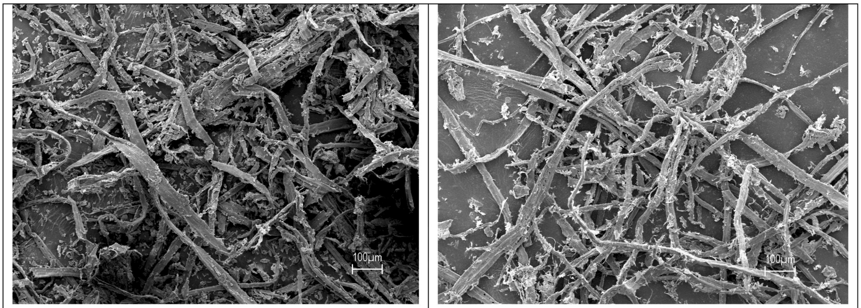
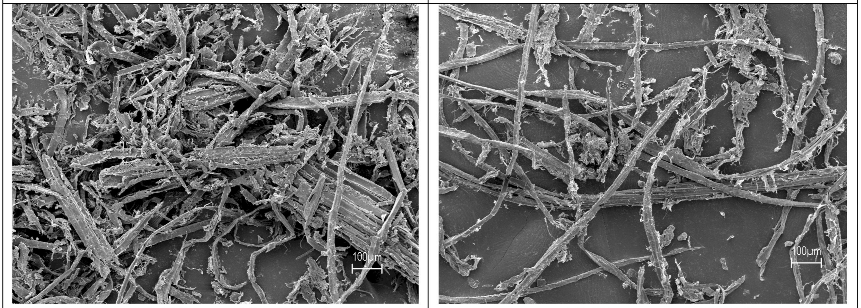


(a) SPORL, pH=1.9

(b) SPORL, pH=4.2



(c) Dilute acid, pH=1.1

(d) Hot-water, pH=5.0

Figure 4. SEM images of lodgepole pine substrates produced from different pretreatment process and then disk milled at solids content of 6% with disk plate gap of 0.76 mm. The pH values were measured from the initial pretreatment liquor.

Certainly, the assumption of cylinder is an oversimplification. Fibers have lumens. A hollow cylindrical fiber with a very thin wall thickness is a more realistic assumption. Ignoring the wall thickness, the specific surface is simply two times the specific surface of a cylinder (Eqs. 3 and (5). When chemical pretreatment such as Kraft pulping is applied, the fiber lumen can collapse and the fiber is more like a ribbon with a rectangular cross section (Figure 4). Tables II and III list the suitable expressions for estimating specific surfaces of different fiber cross-sectional shapes, along with the relative deviations from the cylinder assumption (Eqs. 3 and (5) (*23*). Certainly, the relative deviation in specific surface based on the cylinder model from the actual value is a function of the cross section of a fiber. This can be clearly seen from Table II, and especially from Table III for ribbon type fibers, and fibers having elliptical cross sections with various ratios of major and minor axial dimensions (d and w are the dimensions of the two major axes of the cross section). Fortunately, fibers can freely spin in suspension during wet imaging, and therefore, the probabilities of imaging different orientations of a non-cylindrical fiber are the same. As a result, the relative deviation in the measured mean fiber specific surface from that obtained using the cylinder model (with the diameters equal to the measured projection dimensions d_i as listed in Tables II and III) will be significantly smaller than the deviations listed in Tables II and III, when tens of thousands fibers are measured.

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Table II. Equations to calculate the average (volumetric) specific surface of a substrate using fiber models of different cross sections. The measured width (diameter), *d*, is the image projection dimension.

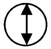
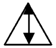
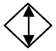
Fiber cross section	**Circle**	**ELT-I**	**ELT-II**	**Square**	**Diamond**
Imaging → Projection direction					
$S_f^{\ V}$	$\frac{4\sum_i n_i d_i (L_i + d_i/2)}{\sum_i n_i L_i \cdot d_i^2}$ $\approx 4 \cdot \frac{\sum_i n_i L_i \cdot d_i}{\sum_i n_i L_i \cdot d_i^2}$ $= \frac{4}{D_{L21}}$	$\frac{6\sum_i n_i d_i (L_i + d_i/3)}{\sum_i n_i L_i \cdot d_i^2}$ $\approx 6 \cdot \frac{\sum_i n_i L_i \cdot d_i}{\sum_i n_i L_i \cdot d_i^2}$ $= \frac{6}{D_{L21}}$	$\frac{4\sqrt{3}\sum_i n_i d_i (L_i + d_i/2/\sqrt{3})}{\sum_i n_i L_i \cdot d_i^2}$ $\approx 4\sqrt{3}\,\frac{\sum_i n_i L_i \cdot d_i}{\sum_i n_i L_i \cdot d_i^2}$ $= \frac{4\sqrt{3}}{D_{L21}}$	$\frac{4\sum_i n_i d_i (L_i + d_i/2)}{\sum_i n_i L_i \cdot d_i^2}$ $\approx 4 \cdot \frac{\sum_i n_i L_i \cdot d_i}{\sum_i n_i L_i \cdot d_i^2}$ $= \frac{4}{D_{L21}}$	$\frac{4\sqrt{2}\sum_i n_i d_i (L_i + d_i/2/\sqrt{2})}{\sum_i n_i L_i \cdot d_i^2}$ $\approx 4\sqrt{2} \cdot \frac{\sum_i n_i L_i \cdot d_i}{\sum_i n_i L_i \cdot d_i^2}$ $= \frac{4\sqrt{2}}{D_{L21}}$
S_f	$\frac{\pi}{2}\frac{\sum_i n_i (d_i^2 + 2 \cdot d_i \cdot L_i)}{m_f}$ $\approx \pi \frac{\sum_i n_i \cdot d_i \cdot L_i}{m_f}$	$\frac{2\sqrt{3}\sum_i n_i d_i (L_i + d_i/3)}{m_f}$ $\approx 2\sqrt{3}\,\frac{\sum_i n_i \cdot d_i \cdot L_i}{m_f}$	$\frac{3\sum_i n_i d_i (L_i + d_i/2/\sqrt{3})}{m_f}$ $\approx 3\frac{\sum_i n_i \cdot d_i \cdot L_i}{m_f}$	$\frac{4\sum_i n_i d_i (L_i + d_i/2)}{m_f}$ $\approx 4\frac{\sum_i n_i \cdot d_i \cdot L_i}{m_f}$	$\frac{2\sqrt{2}\sum_i n_i d_i (L_i + d_i/2/\sqrt{2})}{m_f}$ $\approx 2\sqrt{2}\,\frac{\sum_i n_i \cdot d_i \cdot L_i}{m_f}$
Characteristic Size	$D_{L21} = \frac{\sum_i n_i L_i \cdot d_i^2}{\sum_i n_i L_i \cdot d_i}$	$D_{L21} = \frac{\sum_i n_i L_i \cdot d_i^2}{\sum_i n_i L_i \cdot d_i}$	$D_{L21} = \frac{\sum_i n_i L_i \cdot d_i^2}{\sum_i n_i L_i \cdot d_i}$	$D_{L21} = \frac{\sum_i n_i L_i \cdot d_i^2}{\sum_i n_i L_i \cdot d_i}$	$D_{L21} = \frac{\sum_i n_i L_i \cdot d_i^2}{\sum_i n_i L_i \cdot d_i}$
Relative difference in $S_f^{\ V}$ to Circle		50%	73%	0	41%
Relative difference in S_f to Circle		10%	-5%	27%	-10%

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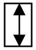
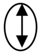
Table III. Equations to calculate the average (volumetric) specific surface of a substrate using columns of different cross sections of rectangles or ellipses with different ratios of width to thickness or major to minor axis, x= d/w

Fiber cross section	**Rectangle** **d/w=x**	**Ellipse** **d/w=x**
Imaging → Projection direction		
$S_f^{\ V}$	$\dfrac{2\sum_i n_i d_i[(x+1)L_i+d_i]}{\sum_i n_i L_i \cdot d_i^{\ 2}}$ $\approx 2(x+1)\cdot\dfrac{\sum_i n_i L_i \cdot d_i}{\sum_i n_i L_i \cdot d_i^{\ 2}} = \dfrac{2(x+1)}{D_{L21}}$	$\dfrac{2\sum_i n_i d_i\{[3(x+1)-\sqrt{(3x+1)(x+3)}]L_i+d_i\}}{\sum_i n_i L_i \cdot d_i^{\ 2}}$ $\approx 2[3(x+1)-\sqrt{(3x+1)(x+3)}]\dfrac{\sum_i n_i d_i L_i}{\sum_i n_i L_i \cdot d_i^{\ 2}}$ $= \dfrac{2[3(x+1)-\sqrt{(3x+1)(x+3)}]}{D_{L21}}$
S_f	$\dfrac{2\sum_i n_i d_i[(x+1)L_i+d_i]/x}{m_f}$ $\approx 2(1+\dfrac{1}{x})\dfrac{\sum_i n_i \cdot d_i \cdot L_i}{m_f}$	$\dfrac{\pi}{2}\dfrac{\sum_i n_i d_i\{[3(x+1)-\sqrt{(3x+1)(x+3)}]L_i+d_i\}/x}{m_f}$ $\approx \dfrac{\pi}{2x}[3(x+1)-\sqrt{(3x+1)(x+3)}]\dfrac{\sum_i n_i d_i L_i}{m_f}$
Characteristic size	$D_{L21} = \dfrac{\sum_i n_i L_i \cdot d_i^{\ 2}}{\sum_i n_i L_i \cdot d_i}$.	$D_{L21} = \dfrac{\sum_i n_i L_i \cdot d_i^{\ 2}}{\sum_i n_i L_i \cdot d_i}$
Relative difference in $S_f^{\ V}$ to circle	$\dfrac{x-1}{2} * 100\%$	$\dfrac{(3x+1)-\sqrt{(3x+1)(x+3)}}{2} * 100\%$
Relative difference in S_f to circle	$\frac{2}{\pi}(2-\frac{\pi}{2}+\frac{1}{x}) * 100\%$	$\dfrac{x+3-\sqrt{(3x+1)(x+3)}}{2x} * 100\%$

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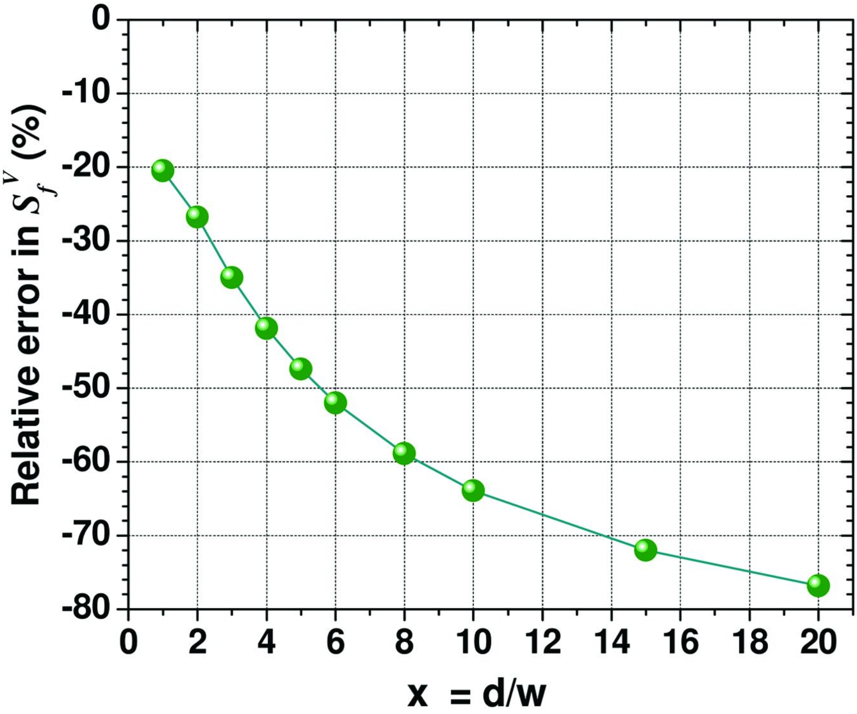

Figure 5. Estimated measurement errors between specific surface measurements of fibers with rectangular cross sections (width d and thickness w) and a circular cross section model using wet imaging techniques .

Figure 5 shows the estimated measurement errors in volumetric specific surfaces between those determined using the cylinder model with equal probability integration (average) over all orientations for ribbon fibers of width *d*, thickness, *w*, and their actual specific surfaces. The results clearly show that the wet imaging measured specific surfaces (integrated over all orientations) are in the same order of magnitude of actual specific surfaces. Most substrate fibers produced for biorefining applications would not be expected to have cross sections with a very large aspect ratio, x, of more than 20, as shown in Figure 4. This is not to deemphasize the errors caused by the geometric shape of fiber cross section using the wet imaging technique described here, but rather to illustrate that the wet imaging technique is probably the best approach currently available to address biomass substrate characterization, a very difficult problem.

Effect of Biomass Substrate Size on Enzymatic Hydrolysis Efficiency

Research has recognized that enzyme accessibility to substrate cellulose is a leading contributing factor to substrate enzymatic digestibility (*24*, *25*). It is also generally understood that substrate surface area directly affects the enzyme accessibility to cellulose and therefore, substrate surface area is a mass transfer limiting factor. However, limited studies have reported the effect of substrate size on cellulose to glucose conversion through enzymatic hydrolysis. In one of those, Rivers and Emert concluded that the particle size of substrates derived from ball milling had no effect on hydrolysis yield in the wet particle-size range of 0.25 to 0.47 mm (*26*). However, others reported that initial wood-chip size can affect cellulose saccharification even when the substrates were obtained after steam explosion of wood chips (*27*, *28*). Particle size also affected enzymatic digestibility of Ammonia Fiber/Freeze Explosion- (AFEX-) pretreated corn stover (*29*). Furthermore, particle size and surface area also affect the rate of hydrolysis (*30*).

Dasari and Berson found that glucose conversion of red-oak sawdust was almost doubled when particle size was reduced from 590–850 to 33–75 μm (*31*). Although the internal pore surface contributed to over 90% of the total surface of a microcrystalline cellulose, substrate hydrolysis efficiency was not affected by the internal pore surface area but rather by the substrate size or external surface (calculated with spherical particle assumption) (*32*). Sangseethong and others suggested that the enzyme-accessible internal pore surface depends on the pore depth dictated by the substrate size. As a result, this study concluded that particle size can affect enzymatic saccharification. Lack of accurate characterization of substrate size as discussed in the previous section can be a contributing factor to the different conclusions in the literature. Another factor may be related to the variability of different substrates used in the literature. It is expected that size effect on enzymatic hydrolysis of cellulose may be more important to untreated lignocellulosic substrates (*4*, *31*) than to pretreated substrates (*3*, *26*) under certain conditions. This will be further elaborated in the following discussions.

Many factors affect enzyme accessibility to substrate cellulose. The total substrate surface area is a representation of potentially accessible area to enzymes. Because of chemical barriers, not all surface areas are "productively" accessible to enzymes. The term "productive" refers to direct interactions between enzymes and cellulose that leads to cellulose hydrolysis. This term is used similarly to the term "non-productive adsorption" of enzymes by non-cellulose such as lignin in lignocellulosic substrate. When surface area is no longer a limiting factor or other factors become dominant, further increases in surface area or reduced substrate size will not significantly affect substrate enzymatic digestibility (SED). When comparing SEDs of fractionated substrates from a spruce thermo-mechanical pulp (TMP), we found that SED increased as substrate specific surface increased up to a certain level, but further specific surface increases did not result in the increase of SED (*4*). This is because the chemical barrier (such as hemicelluloses) becomes a dominant factor, or specific surface is no longer the limiting factor, for the spruce TMP when SED reaches about 20% (*4*). This is also observed from a set of substrates produced from disk milling using different disk plate gaps of the same chemically pretreated lodgepole pine wood chips (*3*). The effect of disk plate gap on enzymatic hydrolysis glucose yield is minimal when the disk plate gap is reduced to less than 1.5 mm, i.e., the substrate is sufficiently fine.

To illustrate this point, Figure 6 plots the SEDs for the two sets of substrates produced from disk milling of dilute acid and SPORL pretreated lodgepople pine wood chips (*3*), respectively. The low constant SEDs of about 40% of the dilute

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acid samples produced using disk plate gaps from 0.38 to 3.8 mm indicate that dilute acid is not able to remove the recalcitrance of lodgepole pine. Chemical barrier is the dominant factor to enzymatic cellulose saccharification. As a result, size reduction to very fine substrates using very small disk plate gaps was not able to increase SED. The high SEDs of over 90% of the SPORL samples produced using disk plate gaps less than 3.8 mm suggest that SPORL effectively removed the chemical barriers, and furthermore, size reduction using a disk plate gap of about 3.8 mm was sufficient to remove the remaining barriers of substrate cellulose accessibility to cellulase. Therefore, the degree of size reduction achieved at a disk plate gap of 3.8 mm was close to maximal. Further reduction of the disk plate gap can no longer improve SED, as SED is already in the 90th percentile. However, increasing the disk plate gap resulted in small reductions in SED in general as expected. Because size-reduction energy consumption often increases with specific surface exponentially (*7*), this suggests that it is not cost effective to produce an extremely fine substrate in size reduction. There may be an optimal degree of size reduction for a given chemically pretreated biomass, as will be further elaborated in the next section.

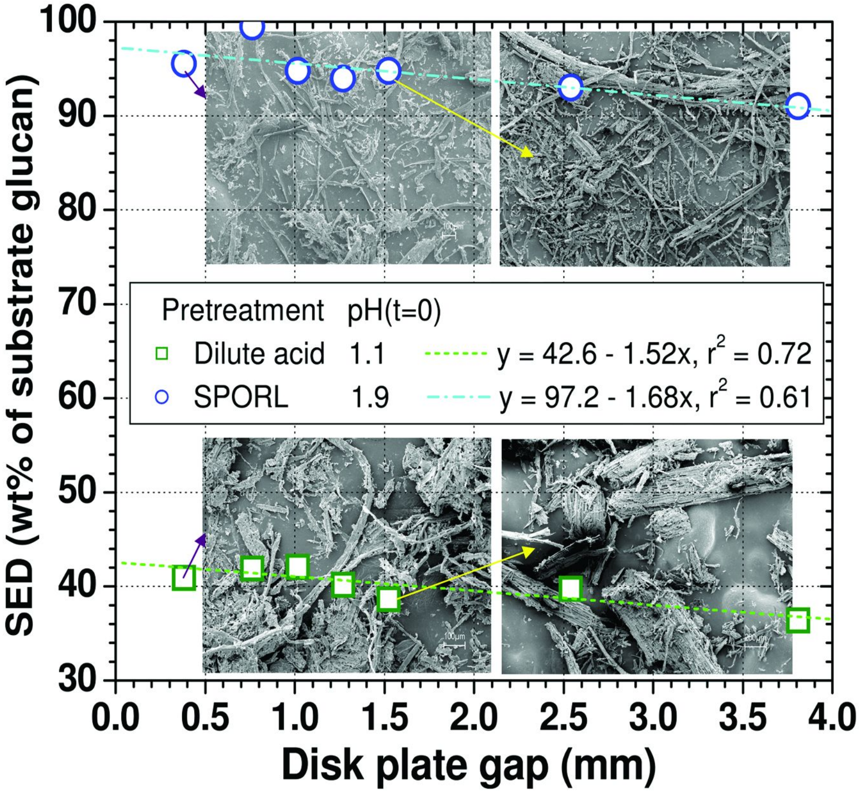

Figure 6. Effects of disk refining disk plate gap on substrate size/morphology and substrate enzymatic digestibility (SED).

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Optimal Degree of Wood-Size Reduction and Size-Reduction Energy Efficiency

The term of "productive" substrate surface area illustrates the limit of size reduction on improving SED. The competing barriers to substrate enzymatic cellulose saccharification suggest there may be an optimal degree of size reduction, beyond which the "surface productivity" of the substrate decreases. The "surface productivity" can be expressed as the enzymatic hydrolysis glucose yield (EHGY) per unit surface area of the substrate, or simply the unit surface glucose yield as defined in our previous study (*4*), i.e.,

$$Surface\ Productivity = \frac{EHGY\ (g)}{Substrate\ surface\ area\ (m^2)} = \frac{EHGY\ (wt\%\ of\ substrate)}{Substrate\ specific\ surface\ (\frac{m^2}{g})} \tag{6}$$

The optimal degree of size reduction, or optimal specific surface, corresponds to the maximal Surface Productivity. Our previous study (*4*) found that the optimal degree of size reduction was about 0.1 (μm^2/μm^3) for a TMP spruce pulp. To achieve energy efficient biomass size reduction, size reduction should be conducted close to this optimal degree, and more specifically, slightly beyond this degree of size reduction because EHGY still increases beyond this degree, although surface productivity decreases.

A size-reduction energy efficiency can be defined as the glucose yield on unit size-reduction energy consumption (*4*), similar to the definition of pretreatment energy efficiency (*2*, *5*),

$$\text{Size} - \text{reduction energy efficiency} = \frac{EHGY\ (kg)}{\text{Size} - \text{reduction energy}\ (GJ)} \tag{7}$$

When comparing the performance of various size-reduction technologies or processes, both the size-reduction energy efficiency defined above along with EHGY should be used. Pretreatment can significantly affect the size-reduction energy efficiency by (1) reducing the energy consumption as discussed early in this chapter and (2) increasing enzymatic hydrolysis glucose yield caused by removing feedstock recalcitrance. One could define the size-reduction efficiency using the total substrate surface area divided by the size-reduction energy consumption. However, this definition does not take the issue of "non-productive surface" into account.

Summary

Wood-size reduction is very energy intensive and can significantly affect the overall energy efficiency of forest biorefinery. The post-pretreatment size-reduction approach can significantly reduce energy consumption for wood size reduction to the level equivalent to those used for herbaceous biomass. The degree of size reduction can not only affect energy consumption in size reduction, but can also affect enzymatic saccharification of biomass substrate. However,

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proper characterization of the size of biomass substrate is very difficult. Wet imaging analysis is probably the best available approach to effectively determine biomass substrate size. The specific surface-derived characteristic length (D_{L21}), fiber length weighted-surface-length mean fiber diameter or width, should be used to characterize biomass substrate. Not all substrate surfaces are productive for enzymatic hydrolysis. The concept of "Surface Productivity" defined as the enzymatic hydrolysis glucose yield (EHGY) on unit substrate surface can be used to describe the effectiveness of size reduction. Competing processes can affect enzymatic hydrolysis. When substrate surface is not the limiting factor to cellulose hydrolysis, further reducing substrate size will not result in improvement in cellulose saccharification. There is an optimal degree of wood size reduction, which should be close to the specific surface corresponding to the maximum "surface productivity."

Acknowledgments

This work was conducted on official government time. It is in the public domain in the United States.

References

1. Perlack, R. D.; Wright, L. L.; Turhollow, A.; Graham, R. L.; Stokes, B.; Erbach, D. C. *Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-Ton Annual Supply*; Oak Ridge National Laboratory, US Dept. of Energy: Oak Ridge, 2005.
2. Zhu, J. Y.; Pan, X. J. Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation. *Bioresour. Technol.* **2010**, *101*, 4992–5002.
3. Zhu, W.; Zhu, J. Y.; Gleisner, R.; Pan, X. J. On energy consumption for size-reduction and yield from subsequent enzymatic sacchrification of pretreated lodgepole pine. *Bioresour. Technol.* **2010**, *101*, 2782–2792.
4. Zhu, J. Y.; Wang, G. S.; Pan, X. J.; Gleisner, R. Specific surface to evaluate the efficiencies of milling and pretreatment of wood for enzymatic saccharification. *Chem. Eng. Sci.* **2009**, *64*, 474–485.
5. Zhu, J. Y.; Pan, X. J.; Zalesny, R. S., Jr. Pretreatment of woody biomass for biofuel production: Energy efficiency, technologies and recalcitrance. *Appl. Microbiol. Biotechnol.* **2010**.
6. Cadoche, L.; Lopez, G. D. Assessment of size-reduction as a preliminary step in the production of ethanol from lignocellulosic wastes. *Biol. Wastes* **1989**, *30*, 153–157.
7. Holtzapple, M. T.; Humphrey, A. E.; Taylor, J. D. Energy-requirements for the size-reduction of poplar and aspen wood. *Biotechnol. Bioeng.* **1989**, *33*, 207–210.
8. Schell, D. J.; Harwood, C. Milling of lignocellulosic biomass: Results of pilot-scale testing. *Appl. Biochem. Biotechnol.* **1994**, *45-46*, 159–168.

9. Mani, S.; Tabil, L. G.; Sokhansanj, S. Grinding performance and physical properties of wheat and barley straws, corn stover and switchgrass. *Biomass Bioenergy* **2004**, *27*, 339–352.
10. Reineke, L. H. *Wood Flour*; USDA Forest Service, Forest Products Laboratory, Madison, WI, 1961.
11. Irvine, G. M. The significance of the glass transition of lignin in the thermomechanical pulping. *Wood Sci. Technol.* **1985**, *19*, 139–149.
12. Tienvieri, T.; Huusari, E.; Sundholm, J.; Vuorio, P.; Kortelainen, J.; Nystedt, H.; Artamo, A., Thermomechanical Pulping. In *Mechanical Pulping*; Sundholm, J., Ed.; Fapet Oy: Jyvaskyla, Finland, 1999; pp 157−221.
13. Mihelich, W. G.; Wild, D. J.; Beaulieu, S. B.; Beath, L. R. Single stage chip refining: Some major operating parameters and their effects on pulp quality. *Pulp Pap. Mag. Can.* **1972**, *73*, 78–82.
14. Alami, R.; Boileau, I.; Harris, G.; Lachaume, J.; Karnis, A.; Miles, K. B.; Roche, A. In *Evaluation of the Impact of Refining Intensity on Energy Reduction in Commercial Size Refiner: The Effect of Primary Stage Consistency*; Int. Mechanical Pulping Conference Ottawa, Ontario, Canada, 1995; Canadian Pulp and Paper Association: Ottawa, Ontario, Canada, 1995; pp 203−211.
15. Lynd, L. R. Overview and evaluation of fuel ethanol from cellulosic biomass: Technology, economics, the environment, and policy. *Annu. Rev. Energy Environ.* **1996**, *21*, 403–465.
16. Yang, B.; Wyman, C. E. Pretreatment: The key to unlocking low-cost cellulosic ethanol. *Biofuels, Bioprod. Biorefin.* **2008**, *2*, 26–40.
17. U. S. DOE. *Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda. A Research Road Map Resulting from the Biomass to Biofuel Workshop Sponsored by the U.S. Department of Energy*; Rockville, MD, 2005.
18. Zhu, J. Y.; Pan, X. J.; Wang, G. S.; Gleisner, R. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresour. Technol.* **2009**, *100*, 2411–2418.
19. Kenealy, W.; Horn, E.; Houtman, C. J. Vapor phase diethyl oxalate pretreatment of wood chips: Part 1. Energy savings and improved pulps. *Holzforschung* **2007**, *61*, 223–229.
20. Wang, G. S.; Pan, X. J.; Zhu, J. Y.; Gleisner, R. Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) for robust enzymatic saccharification of hardwoods. *Biotechnol. Prog.* **2009**, *25*, 1086–1093.
21. Lefebvre, A. H. *Atomization and Sprays*; Hemisphere Publishing Corp.: New York, 1989.
22. Sowa, W. A. Interpreting mean drop diameter using distribution moments. *Atomization Sprays* **1992**, *2*, 1–15.
23. Wang, G. S.; Pan, X. J.; Zhu, J. Y.; Gleisner, R. L. Sulfite Pretreatment for Biorefining Biomass. U.S. Patent Application 12/425,773, April 2009; U.S. Patent Publication US 2009/0298149 A1, December 3, 2009.
24. Jeoh, T.; Ishizawa, C. I.; Davis, M. F.; Himmel, M. E.; Adney, W. S.; Johnson, D. K. Cellulase digestibility of pretreated biomass is limited by cellulose accessibility. *Biotechnol. Bioeng.* **2007**, *98*, 112–122.

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25. Rollin, J. A.; Zhu, Z.; Sathitsuksanoh, N.; Zhang, Y.-H. P. Increasing cellulose accessibility is more important than removing lignin: A comparison of cellulose solvent-based lignocellulose fractionation and soaking in aqueous ammonia. *Biotechnol. Bioeng.* **2011**, *108*, 22–30.
26. Rivers, D. B.; Emert, G. H. Lignocellulose pretreatment: A comparison of wet and dry ball attrition. *Biotechnol. Lett.* **1987**, *9*, 365–368.
27. Cullis, I. F.; Saddler, J. N.; Mansfield, S. D. Effect of initial moisture content and chip size on the bioconversion efficiency of softwood lignocellulosics. *Biotechnol. Bioeng.* **2004**, *85*, 413–421.
28. Ballesteros, I.; Oliva, J. M.; Navarro, A. A.; Gonzalez, A.; Carrasco, J.; Ballesteros, M. Effect of chip size on steam explosion pretreatment of softwood. *Appl. Biochem. Biotechnol.* **2000**, *84-86*, 97–110.
29. Chundawat, S. P. S.; Venkatesh, B.; Dale, B. E. Effect of particle size based separation of milled corn stover on AFEX pretreatment and enzymatic digestibility. *Biotechnol. Bioeng.* **2007**, *96*, 219–231.
30. Mooney, C. A.; Mansfield, S. D.; Beatson, R. P.; Saddler, J. N. The effect of fiber characteristics on hydrolysis and cellulase accessibility to softwood substrates. *Enzyme Microb. Technol.* **1999**, *25*, 644–650.
31. Dasari, R. K.; Berson, R. E. The effect of particle size on hydrolysis reaction rates and rheological properties in celluslosic slurries. *Appl. Biochem. Biotechnol.* **2007**, *137*, 289–299.
32. Sangseethong, K.; Meunier-Goddik, L.; Tantasucharit, U.; Liaw, E. T.; Penner, M. H. Rationale for particle size effect on rates of enzymatic saccharification of microcrystalline cellulose. *J. Food Biochem.* **1998**, *22*, 321–330.

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Chapter 5

Chemistry and Reactions of Forest Biomass in Biorefining

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This chapter summarizes the chemistry and reactions of forestry biomass during biorefining. Broad classification of forest biomass, chemical composition, and chemistry of macromolecular cell wall components (cellulose, hemicellulose, and lignin) are addressed. Major chemical reactions of the macromolecular components during biorefining are discussed briefly, which includes acid-, alkali-, and enzyme-catalyzed hydrolysis of cellulose and hemicellulose, and degradation/condensation of lignin in acid, alkali, sulfite, and organosolv media. The influence of substrate features, such as crystallinity, degree of polymerization, surface area, hemicellulose, and lignin structure and distribution, on enzymatic reactions of biomass is also discussed.

1. Introduction

Forest biomass is defined as any plant or tree material produced by forest growth. In general forestry occupies an ambiguous position in environmental debates. Forests are a source of energy through the conversion of woody biomass into convenient solid, liquid or gaseous fuels to provide energy for industrial, commercial or domestic use. It is estimated that forest and agricultural lands alone (the two largest potential biomass sources) could produce enough biofuels to meet more that one-third of the current demand for transportation fuels (*1*). Increased attention to federal renewable energy and climate change policies, forests are increasingly turned to as a source of energy (*2*). Much of the forest biomass is currently used as a raw material in the manufacturing and refining of

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

traditional types of wood products, such as timber, fiberboard, pulp, and paper. The conversion of forestry biomass to fuels and chemicals has long been a goal of the forest products industry. A primary challenge of the near future is the development of alternative energy sources to make this nation less dependent on fossil fuel. Increasing the use of forest biomass for energy production has been suggested as one approach of meeting this goal (*3*).

2. Classification of Forest Biomass

A forest may be defined as a collection of trees occupying a certain ground area and forming an ecosystem together with many other living and dead organisms in an inorganic environment includes the mineral soil and atmosphere (*4*). Trees belong to seed-bearing plants (*Spermatohytae*), which are subdivided into gymnosperms (*Gymnospermae*) and angiosperms (*Angiospermae*). Coniferous woods or softwoods belong to the first-mentioned category and hardwoods to the second group (*5*, *6*). Softwoods generally do not have flowers and use cones for seed reproduction. Its cellular structure is simple and 90-95% of the cells are longitudinal tracheids (Figure 1A). Longitudinal tracheids function in water conduction and support. The limited number of cell types makes softwoods more difficult to differentiate from one another. Examples of softwoods include pines, spruces, firs and hemlocks. Hardwoods use flowers to pollinate for seed production. Its structure is more complex than softwood structure, and varies considerably between species (Figure 1B).

The majority of hardwood volume is composed of fiber cells that offer structural support to the stem. The major difference between hardwoods and softwoods is the presence of vessel elements, or pores, that exist in hardwoods only. The main function of vessel element is water conduction. Oaks, maples, birches are some examples of hardwoods (*7*).

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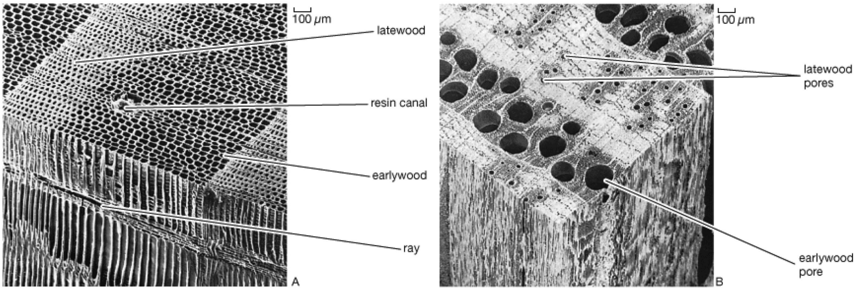

Figure 1. A) Transverse plane of softwood (Pinus sylvestris) with a whole annual ring, B) Transverse plane of a ring porus hardwood (Quercus robur) (adapted from ref. (6)).

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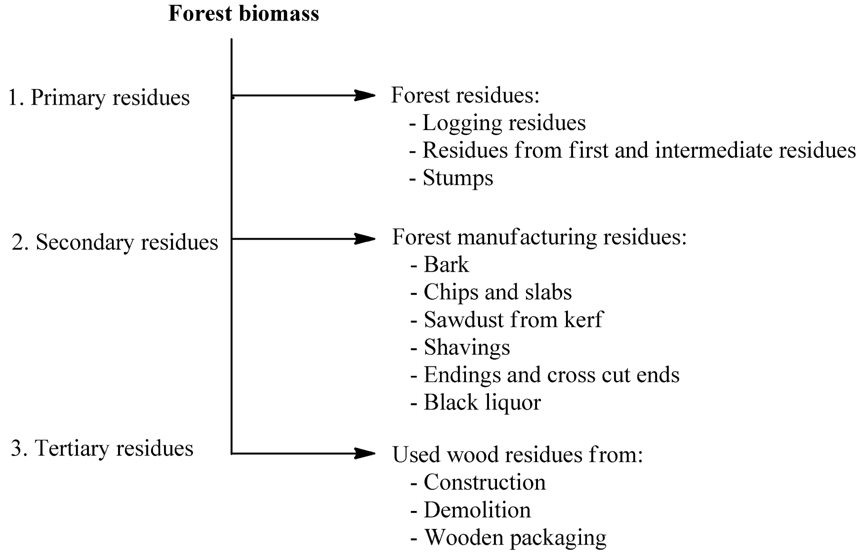
Forest biomass
1. Primary residues
Forest residues:
- Logging residues
- Residues from first and intermediate residues
- Stumps
2. Secondary residues
Forest manufacturing residues:
- Bark
- Chips and slabs
- Sawdust from kerf
- Shavings
- Endings and cross cut ends
- Black liquor
3. Tertiary residues
Used wood residues from:
- Construction
- Demolition
- Wooden packaging

Figure 2. Schematic representation of broad classification of forest biomass. Based on ref. (10).

Forest residues typically refer to those parts of trees such as tree tops, branches, small-diameter wood, stumps and dead wood, as well as undergrowth and low-value species. The forest residues can be divided into three groups: primary, secondary and tertiary residues (*8–10*). The main sources of primary forest residues and thinnings are the logging residues that are a by-product of conventional forest operations (Figure 2). Industrial residues or secondary residues are by-products of forest industrial processes, including bark, sawdust, shavings and chips, endings, cross-cut ends, and black liquor from wood pulping. Recycled wood or tertiary residues which are another sources of woody biomass for energy consist predominantly of by-products of demolition, construction and packaging process, offering a significant and inexpensive source of energy (*11*, *12*).

3. Chemical Composition of Forestry Biomass

The chemical substances composing of forest biomass (wood) can be broadly divided into two classes: macromolecular substances and lower molecular weight substances of both simple and complex chemical structure, collectively known as extractives (*7*). The chemical composition of wood cannot be defined precisely for a given tree species or even for a given tree. Chemical composition varies with tree part (root, stem, or branch), type of wood (softwood, hardwood), climate, and soil condition (*13*). Ordinary chemical analysis can distinguish between hardwoods and softwoods. Unfortunately, such techniques cannot be used to identify individual tree species because of the variation within each species and the similarities among any species (*14*). Further identification is possible with detailed chemical analysis of extractives (chemotaxonomy). A short introduction

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to the chemical components of forest biomass (wood) follows the general scheme (Figure 3).

Forestry biomass (wood) is a composite material composed of fibers of cellulose (40-50%) and polyoses or hemicelluloses (20-30%) held together by a third substance called lignin (20-30%). Minor amounts of extraneous materials (low molecular weight components), mostly in the form of organic extractives and inorganic minerals (ash), are also present (4-10%) in wood (*15*). Other polymeric substances are also present in small amounts as starch, protein, and pectin derivatives (*7*). Overall, wood has an elemental composition of about 50% carbon, 6% hydrogen, 44% oxygen, and trace amounts of nitrogen and several metal ions. The proportions and chemical composition of lignin and polyoses differ in softwoods and hardwoods (Table 1), while cellulose is a uniform component of all woods.

3.1. Macromolecular Substances

Cellulose, the major chemical component of cell wall and making up approximately one half both softwoods and hardwoods, is composed of linear chains of D-glucose linked by β-1,4-glycosidic bonds. Because of its chemical and physical properties as well as its supramolecular structure it can fulfill its function as the main structural component of the plant cell walls. Softwood cellulose fibers measure from about 2 to 4 mm in length, and hardwood fibers range from about 0.5 to 1.5 mm (*16*).

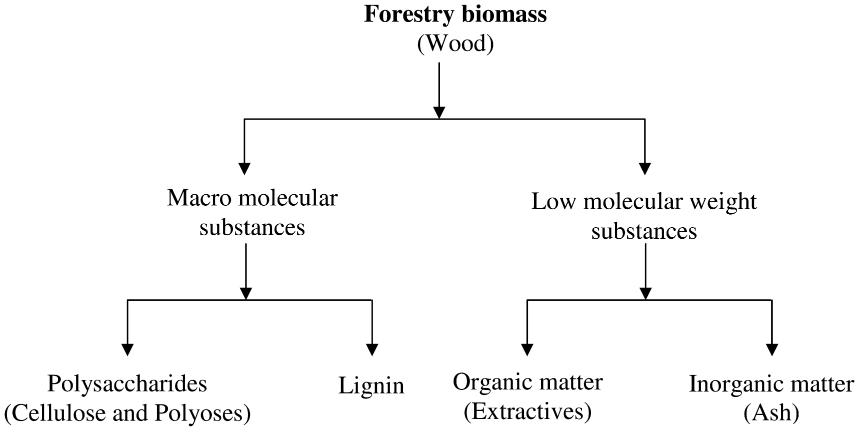
Forestry biomass
(Wood)
Macro molecular substances
Low molecular weight substances
Polysaccharides (Cellulose and Polyoses)
Lignin
Organic matter (Extractives)
Inorganic matter (Ash)

Figure 3. General scheme of chemical components from forestry biomass. Based on ref. (14).

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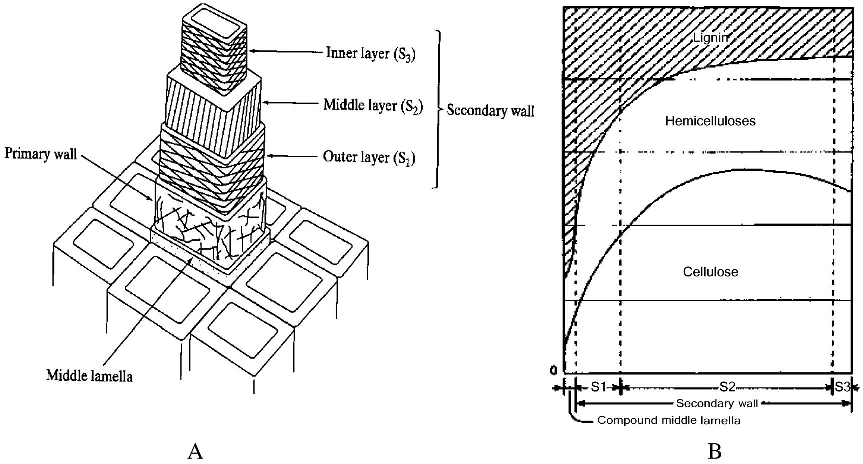

Figure 4. A) Idealized microfibril cell wall construction; B) Distribution of macromolecular components within cell wall layers of coniferous wood (adapted from ref. (21)).

Polyoses (hemicelluloses) are complex, branched carbohydrate polymeres that are formed from different monomeric sugars attached through differen linkages. They are intimately associated with cellulose and appear to contribute as a structural component in the plant. The main feature that differentiates hemicellulose from cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars. Hemicelluloses are generally classified according to the types of monosaccharides present (*17*). These monosaccharides include hexoses (glucose, mannose, and galactose), pentoses (xylose and arabinose), and uronic acids (e.g., 4-*O*-methylglucuronic, D-glucuronic, and D-galactouronic acids). The main hemicelluloses of softwood are galactoglucomannans and arabinoglucuronoxylan, while in hardwood is glucuronoxylan (*18*).

Lignin is the third macromolecular component of wood representing 20-30% of its total weight. This macromolecule plays a vital role in providing mechanical support to bind plant fibers together and protecting wood from microbial attack. Lignin has been described as a random, three-dimensional network polymer comprised of variously linked phenylpropane units. In contrast to softwood lignins, composed mostly of guaiacyl-type lignin, hardwood lignins consist of syringyl and guaiacyl units. In general, there is more lignin in softwoods than in hardwoods (*19*). From the morphological point of view lignin is an amorphous substance located in the compound middle lamella as well as in the secondary walls.

It is important to recognize that cellulose, hemicellulose, and lignin occur in each layer of the cell wall. The cell wall is composed of a thin, outer primary wall (P) and a much thicker, inner secondary wall (*15*, *20*). The secondary wall is composed of the S_1 layer, a thick middle S_2 layer, and an inner S_3 layer, as illustrated in Figure 4A. All regions of the cell wall contain varying amounts of components (cellulose, hemicellulose, and lignin). Figure 4B illustrates the distribution of macromolecular components within various cell wall layers of softwood (coniferous wood). The middle lamella contains mostly lignin, with small amounts of cellulose and hemicellulose. The primary cell wall has proportionally more cellulose and hemicellulose than the middle lamella, but less than the secondary wall. The S_1 layer is mostly filled by lignin, and the rest comprises with cellulose and hemicellulose. The layer S_2 mostly contains cellulose followed by hemicellulose and lignin, and finally the S_3 still contains cellulose, but with a large fraction of hemicellulose and low lignin (*21*).

3.2. Accessory Substances (Low Molecular Weight)

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Besides the major cell wall components of forest biomass there are numerous compounds which are called accessory or extractive materials. Though these components contribute only a few percent to the wood mass, they may have a great influence on the properties and processing qualities of woods. A simple classification can be made by dividing the low molecular weight substances into organic and inorganic matter. The low molecular weight substances belong to very different classes of chemical compounds. The organic matter is commonly called extractives and the inorganic part is summarily obtained as ash.

Extractives (Organic Matter)

A diverse array of relatively low molecular weight compounds which often give wood color and can protect wood from decay are known as extractives. Extractives are a variety of organic compounds including aromatic (simple and complex phenolic, terpenes) compounds, aliphatic acids (fats, waxes, and essential oils), alkaloids (glycosides), proteins, simple sugars, mucilages, gums, resins, and saponins. Many of these function as intermediates in tree metabolism, as energy reserves, or as part of the tree's defense mechanism against microbial attack. Extractives may be hydrophobic or hydrophilic; may be soluble in organic solvents or water. The extractives content of trees is typically less than 10%, and the distribution of extractives varies by species, as well as location within an individual tree (*22*, *23*).

Aromatic (phenolic) compounds, the most important substance of this group are tannins. Tannin compounds can be divided into three major classes: i) hydrolysable tannins, ii) non-hydrolysable tannins or condensed tannins, and iii) pseudotannins. Hydrolysable tannins are mixtures of polygalloyl glucose and/or poly-galloy quinic acid derivatives containing 3 to 12 gallic residues per molecule. This tannin is hydrolyzed by weak acids or weak bases to produce carbohydrate and phenolic acids. Condensed tannins (also called as catechol tannins), are polymers of flavonoid units joined by carbon-carbon bonds, which are not susceptible to being cleaved by hydrolysis. Pseudo-tannins are low molecular weight compounds associated with other compounds, they do not respond to the goldbeater's skin test but it possesses other properties like tannin (*24*). Other phenolic substances include stilbenes and lignans, and their derivatives.

Terpenes are cyclic compounds, which characterizes the odor of many woods, leaves, seeds, flowers, and roots. Chemically, terpenes are grouped together because of their distinctive carbon skeleton. It consists of a basic five carbon isoprene unit (2-methyl-1,3-butadiene). Terpenes are generally are composed of two, three, four, or six isoprene units. These are called monoterpenes, sesquiterpenes, diterpenes, and triterpenes, respectively (*25*). Most of the terpenes are obtained from pine trees.

Saturated and unsaturated higher fatty acids are found in wood as glycerides-esters of glycerol. Among the glycerides, trimesters (triglycerides) are dominant. Waxes are complex mixtures of aliphatic compounds, and a majority of these compounds are wax esters composed of fatty acids and fatty alcohols, hydrocarbons and derivatives (*26*, *27*).

Alcohols occur very widely distributed in the plant kingdom, but not in the free condition. It has been claimed that ethyl alcohol has been found in combination with other substances, the compounds in which they occur are known as esters or ethereal salts. Most of the aliphatic alcohols in wood occur as ester components (*28*). In addition, sterols may also present as fatty acid esters or as glycosides (*29*).

Inorganic Matters

The chemical composition of inorganics in a tree depends to some extent on the environmental conditions under which the tree grew, especially on the soil characteristics and parent rock (*30*). The higher concentration was found in cambium in comparison to the adjacent bark and wood. Bark is generally richer in inorganic constituents than the wood (*7*). The mineral constituents are usually studied by analysis of ash which remains when burning the organic matter at high temperatures. Among more than 50 elements detected in wood by neutron activation analysis are Na, Mg, Al, Si, P, S, Cl, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, and Pb (*27*, *31*). The major element is calcium (about 80%); potassium and magnesium are the other predominating elements. The content of other elements is less than 1% of the total.

These elements probably occur in wood and bark as salts of oxalates, carbonates, phosphates, silicates, sulfates, or inorganic moiety bound to cell wall components (*40*, *41*). Ash content is usually more than 5% of bark from deciduous trees and less than 5% of bark from conifers; but the content does not exceed 1% of the wood, often less than 0.5% (*40*).

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Table 1. Chemical composition of some representative softwoods and hardwoods (%)

Scientific name	*Common name*	*Carbohydrate* *Cellulose*	*Galactoglucomannan*	*Arabino-O-4-methyl-glucuronoxylan*	*Arabinogalactan*	*Uronic unhydride*	*Lignin*	*Tannin*	*Aliphatic acids & proteins*	*Resins, Gums*	*Ash*	*Ref.*
Softwoods												
Abies alba	White spruce	42.3	12.0	11.5	1.4	3.4	28.9	0.3	0.7	2.0	0.8	(*32–34*)
Araucaria canadensis	Parana pine	44.3	11.0	4.5	1.8	4.0	29.5	0.3	0.7	4.0	1.4	(*32–34*)
Pinus sylvestris	Red pine	52.2	11.0	3.1	8.2	5.6	26.3	0.3	0.7	4.0	0.4	(*33–35*)
Pseudotsuga menziesii	Douglas Fir	50.4	11.0	7.5	4.7	3.8	27.2	0.4	2.6	2.0	0.2	(*33*, *34*, *36*)
Thuja plicata	Red cedar	47.5	8.0	11.4	1.2	4.2	32.5	0.7	0.7	1.6	0.3	(*33*, *34*, *37*)
Tsuga Canadensis	Hemlock	44.0	11.0	6.5	0.6	3.3	33.0	0.7	2.2	1.1	0.2	(*33*, *34*, *37*)

Hardwoods	*Common name*	*Cellulose*	*Glucomannan*	*O-Acetyl-O-4-methyl- glucuronoxylan*	*Arabinogalactan*	*Uronic unhydride*	*Lignin*	*Tannin*	*Aliphatic acids & proteins*	*Resins, Gums*	*Ash*	*Ref.*
Fagus sylvatica	Beech	36.0	2.7	23.5	1.3	4.8	30.9	0.8	3.2	2.0	0.4	(*32–34*)
Fraxinus excelsior	Ash	46.0	1.9	16.4	1.7	-	22.0	0.9	0.5	0.6	0.4	(*33*, *34*, *37*)

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Hardwoods	*Common name*	*Cellulose*	*Glucomannan*	*O-Acetyl-O-4-methyl- glucuronoxylan*	*Arabinogalactan*	*Uronic unhydride*	*Lignin*	*Tannin*	*Aliphatic acids & proteins*	*Resins, Gums*	*Ash*	*Ref.*
Juglans nigra	Walnut	40.8	-	12.6	-	-	29.1	0.6	4.4	5.0	0.8	(*32–34*)
Swietenia macrophylla	Mahogany	43.9	-	16.0	-	-	28.2	1.9	5.1	1.0	1.1	(*33*, *34*, *38*)
Populus alba	White poplar	49.0	-	25.6	-	-	23.1	0.6	2.0	2.4	0.2	(*33*, *34*, *39*)
Quercus robur	Oak	41.1	3.3	22.2	1.6	4.5	19.6	1.2	2.0	0.4	0.3	(*32–34*)
Ulmus procera	Elm	43.0	3.2	21.8	1.4	3.6	27.3	0.6	1.7	1.7	0.8	(*32–34*)

Other polymeric substances are also present in small amounts as starch and pectin derivates. Pectin, which is not considered, is present in minor increments (1% - 4%). Pectin is a complex carbohydrate in the primary cell wall, is closely associated with lignin, and is easily lost during delignification reactions (*42*). The pectin content of wood is very variable, the youngest part, the cambium, having the most. Pectin occurs in wood in three forms; (i) dissolved in the cell sap, (ii) as insoluble pectic substances in the middle lamella, and (iii) as insoluble protopectins probably intermixed with cellulose (*43*). Starch in wood is detectable only in very small amounts, formed in living cells; the parenchyma cells contain it in granular from stored as reserve food material. Albumin (protein) account for at most 1% of wood, but are mainly found in the non-wooden parts of the stem. The protein content of different parts of the plant is very variable (*44*).

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4. Chemical Structure of Macromolecular Cell Wall Components

4.1. Cellulose

Cellulose is defined as a macromolecule, a linear condensation polymer consisting of D-anhydroglucopyranose joined together by β-1,4-glycosidic bonds (Figure 5). Cellulose molecules, because of their unique strucutre, have a strong tendency to form extensive hydrogen bonds. Two intra-chain hydrogen bonds exist between O3-H3···O5 and between O2-H2···O6, along with one inter-chain hydrogen bond between O6-H6···O3 and that these hydrogen bonds are responsible for the structure of cellulose I (*45*). As a result of these hydrogen bonds and van der Waals forces, cellulose molecules could align together in a highly ordered fashion to form a crystalline region, whereas the less ordered molecules being non-crystalline are usually termed amorphous materials. These hydrogen bonds are considered to be important to the mechanical and chemical properties of cellulose. According to reviews (*46–48*), it is known that cellulose exists in at least four polymorphic crystalline forms, of which the structures and properties of cellulose I (native cellulose) and cellulose II (regenerated cellulose and mercerized cellulose) have been most extensively studied.

4.2. Polyoses (Hemicellulose)

In contrast to cellulose, hemicellulose is a branched carbohydrate copolymer in which the monomers are different sugars. The sugar units (anhydro-sugars) making up the polyoses can be subdivided into groups such as pentoses, hexoses, hexuronic acids, and deoxy-hexoses (*7*). The main chain of a polyose can consist of only one sugar (homopolymer), i.e. xylan, or of two or more sugars (heteropolymer), i.e. glucomannan. Some of the sugars are always or sometimes side groups of a main chain (backbone), i.e. 4-*O*-methylglucuronic acid and galactose (Figure 6). The molecular weight of hemicellulose is significantly lower than that of cellulose (*49*). The principal pentose sugar in hemicellulose is β-D-xylopyranose, which has only the positions 2- and 3-carbons available for *O*-linked susbtitution when β-(1,4) linked as in xylan. This polymer is the simplest general representation of a normal hemicellulose. The other common fiver carbon sugar is arabinose, which is disticnt that it forms a furanose ring structure (*50*). In general, arabinose is linked (1-2) or (1-3) to the xylose with variying degrees in arabinoxylan and similarly arabinose is linked (β-3- and β-6-) with variying amounts with galactose in arabinogalactan (*28*, *51*).

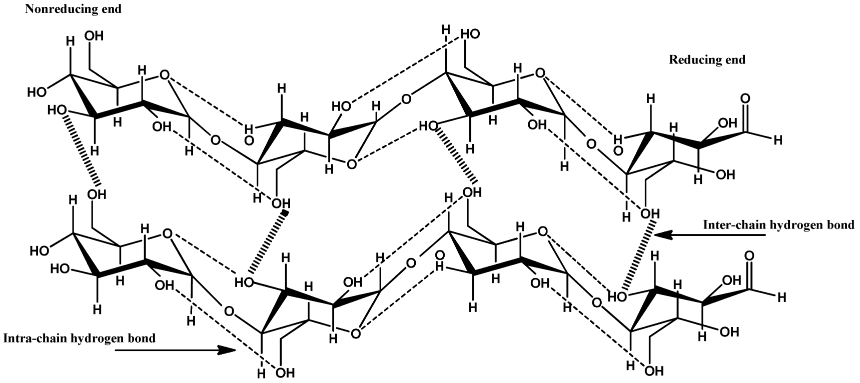
Nonreducing end
Reducing end
Inter-chain hydrogen bond
Intra-chain hydrogen bond

Figure 5. The partial molecular structure of cellulose with inter- and inter-chain hydrogen bonding (from ref. (66)).

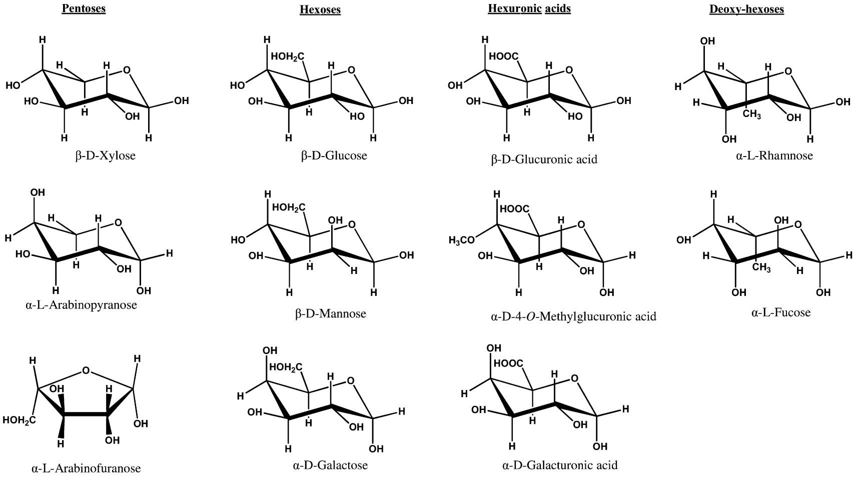
Pentoses
Hexoses
Hexuronic acids
Deoxy-hexoses
β-D-Xylose
β-D-Glucose
β-D-Glucuronic acid
α-L-Rhamnose
α-L-Arabinopyranose
β-D-Mannose
α-D-4-O-Methylglucuronic acid
α-L-Fucose
α-L-Arabinofuranose
α-D-Galactose
α-D-Galacturonic acid

Figure 6. General chemical structure of sugar component of polyoses (from ref. (7)).

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The postion 1-carbon is always the anomeric carbon and must be used for the glycosidic linkage. Hemicelluloses also contain the aldohexoses glucose, mannose, and galactose, which also form pyranose ring strucutres. With these sugars, the glycosisdic linkage is always on the 1-carbon as a β-bond and the available sites are the 2-, 3-, and 6-carbons for derivation. Hemicelluloses are usually hydrogen bound to cellulose, as well as to other hemicelluloses, which helps stabilize the cell-wall matrix and renders the cell wall insouble in water (*52*). Table 2 illustrates the structure of polyses from forest biomass (*53*, *54*). Softwoods and hardwoods differ not only in the precentages of total polyoses but also in the percentages of individual polyoses and composition of these polyoses (*5*).

The main types of hemicellulose in most softwoods are arabinoglucoronxylans and galactoglucomannans, with the exception of larch trees, which contain a significant amount of arbinogalactan, while the hemicellulose in hardwood is predominantly glucuronoxylan (*42*).

Table 2. Structure of polyoses from forest biomass (from ref. (*5*))

Polysaccharide	*Source*	*Percent-age*	*Compositon*[a]	*Chemcial Linkages*
Arabinoxylan	Grasses	20-40	β-D-Xyl*p*	1→4
			α-L-Ara*f*	1→3, 1→2,3
			4-*O*-Me-α-D-Glc*p*A	1→2
			Feruleyl	-
			p-Coumaroyl	-
			O-Acetyl	-
O-Acetyl-4-*O*-methyl-glucuronoxylan	Hardwoods	10-35	β-D-Xyl*p*	1→4
			4-*O*-Me-α-D-Glc*p*A	1→2
			O-Acetyl	
Glucomannan	Hardwoods	3-5	β-D-Man*p*	1→4
			β-D-Glc*p*	1→4
Galactoglucomannan (water soluble)	Softwoods	5-10	β-D-Man*p*	1→4
			β-D-Glc*p*	1→4
			α-D-Gal*p*	1→6
			O-Acetyl	-

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Table 2. (Continued). Structure of polyoses from forest biomass (from ref. (*5*))

Polysaccharide	*Source*	*Percent-age*	*Compositon*[a]	*Chemcial Linkages*
Galactoglucomannan (alkali soluble)	Softwoods	10-15	β-D-Man*p*	1→4
			β-D-Glc*p*	1→4
			α-D-Gal*p*	1→6
			O-Acetyl	-
Arabino-4-*O*-methyl glucuronoxylan	Softwoods	10-15	β-D-Xyl*p*	1→4
			4-*O*-Me-α-D-Glc*p*A	1→2
			α-L-Ara*f*	1→3, 1→6
Arabinogalactan	Larchwood	10-20	β-D-Gal*p*	1→3
			α-L-Ara*f*	1→3, 1→6
			β-L-Ara*f*	1→3
			β -D-Glc*p*A	1→6

[a] Sugar units: β-D-glucopyranose (Glc*p*); β-D-mannopyranose (Man*p*); β-D-galactopyranose (Gal*p*); β-D-xylopyranose (Xyl*p*); 4-*O*-methyl-α-D-glucopyranosyluronic acid (Glc*p*A); α-L-arbinofuranose (Ara*f*)

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4.3. Lignin

Lignin is the second most abundant biological material on the planet, and comprises 20-30% of the dry weight of woods. This macromolecule plays a vital role in providing mechanical support to bind plant fibers together. Lignin also decreases the permeation of water through the cell walls of the xylem, thereby playing an intricate role in the transport of water and nutrients (*5*, *42*). Lignin is a highly branched and cross-linked polymer based on oxygenated phenylpropane units. Chemically, lignin is composed of three main types of monolignols or phenylpropane units, as illustrated in Figure 7.

In general, guaiacyl lignin is found in softwoods (which has a methoxyl group on the C-3 position), while syringyl lignin is present in hardwoods (which has methoxyl groups on both the C-3 and C-5 position). *p*-Coumaryl units exist in grass lignin (*55*). The phenylpropane units of lignin (syringyl, guaiacyl and *p*-hydroxyl phenylpropane) are bonded together by a set of linkages (as summarized in Table 3) to form a very complex matrix. The by far most important bond between monolignols is the β-*O*-4 linkage, which counts for 50-60% of total lignin linkages (*59*).

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Table 3. Common linkages in softwood and hardwood lignin structure (from ref. (*56*))

Linkage type	*% of total linkage*	
	Softwood (spruce)	*Hardwood (beech)*
Arylglycerol- β-aryl ether (β-*O*-4)	50	60
Noncyclic benzyl aryl ether (α-*O*-4)	2-8	7
Phenylcoumaran (β-5)	9-12	6
Biphenyl (5-5)	10-11	5
Diaryl ether (5-*O*-4)	4	7
1,2-diaryl propane (β-1)	7	7
Linked through side chains (β- β)	2	3
In glyceraldehydes-2-aryl ether	2	2
In structures condensed in 2- or 6 positions	3	2.5
In biphenyl	11	4.5
In diphenyl ether	4	6.5
In quinine ketal structures	Traces	-

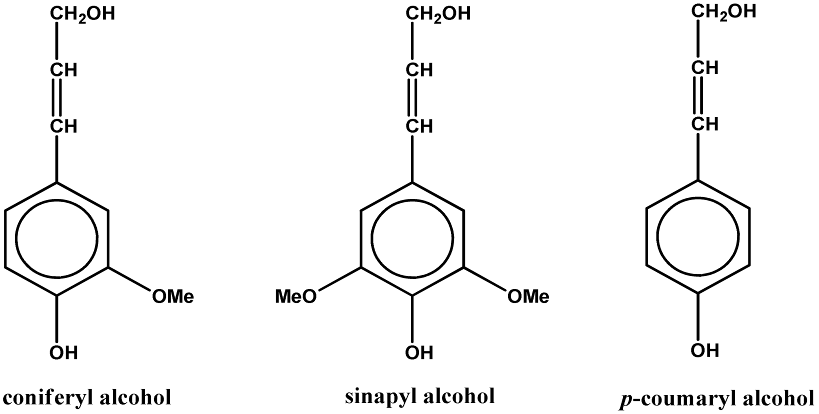
CH2OH
CH
CH
OMe
OH
coniferyl alcohol
CH2OH
CH
CH
MeO
OMe
OH
sinapyl alcohol
CH2OH
CH
CH
OH
p-coumaryl alcohol

Figure 7. Chemical structure of lignin monomeric building units.

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

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Table 4. Functional groups of softwood and hardwood lignin

Functional groups	*Functional groups / 100 monomer units*		*Ref.*
	Softwood (Spruce MWL)	*Hardwood (Aspen wood meal)*	
Methoxyl	96	150	(*57*)
Phenolic hydroxyl	28	12	(*57*)
Aliphatic hydroxyl	35	45	(*57*)
Carbonyl	19	89	(*58*)
Carboxyl	2	3	(*58*)

The most important chemical reactions for delignification in pulping, bleaching, chemical pretreatment, and biological lignin degradation occurs at this bond (β-*O*-4). Carbon-carbon linkages (5-5, β-5, β-1, β-β, and 5-*O*-4) are stable in regular chemical reactions, which is one of the reasons why softwood lignin is more difficult to destruct than hardwood lignin (*60*). Lignin comprises a variety of functional groups (Table 4), such as hydroxyl, methoxyl, carboxyl, and carbonyl, which play a very important role in reactions of delignification and modification and in the interaction of lignin with other substances such as enzymes during the enzymatic saccharification of lignocellulosic substrates (*55*, *61*).

5. Chemical Reactions of Macromolecular Components during Biorefining

5.1. Reactions of Cellulose and Hemicellulose

Acidic Hydrolysis

The most common method of converting cellulosics to useful products is a straightforward hydrolysis to component monosaccharides. The choices for this procedure are either acidic/alkaline hydrolysis or enzymatic hydrolysis. Treatment of lignocellulosic biomass with dilute sulfuric acid is primarily used as a means of hemicellulose hydrolysis/removal and a pretreatment for enzymatic hydrolysis of cellulose (disrupts the lignocellulosic composite). Sulfuric acid is the most investigated acid for the hydrolysis of lignocellulose (*62*), although other acids such as hydrochloric acid have also been used (*63*). Acid hydrolysis can be divided into two groups: a) concentrated-acid hydrolysis and b) dilute-acid hydrolysis. Acid hydrolysis is the essential and most typical degradation reaction of glycosides, di-, oligo- and polysaccharides. The reaction pattern of acid hydrolysis of cellulose by dilute acid is represented in equation (1) by the sequential first-order reaction (*64*, *65*):

$$\text{Cellulose} \xrightarrow{k_1} \text{Glucose} \xrightarrow{k_2} \text{Decomposed products} \qquad (1)$$

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The rate constants of the hydrolysis reactions are represented by an Arrhenius equation (2) in which the per-exponential factors includes the acid concentration term to account for the effect of acid as well as the temperature:

$$k_i = k_{0i}\left[A\right]^{ni} \exp\left(-E_i / RT\right) \tag{2}$$

where i = 1 (hydrolysis reaction), i = 2 (decomposition reaction), n_i = acid exponent, [A] = concentration of sulfuric acid, E_i = activation energy. Wyman et al. (*66*) proposed that at elevated temperatures, the kinetic pattern of hemicellulose hydrolysis can be expressed in a manner similar to that of cellulose hydrolysis (i.e. sequential first order reactions in series).

The β-1,4-glycosidic linkage in cellulose is especially susceptible to acid-catalyzed hydrolytic attack leading to molecular degradation, the extent of which depends on the interaction conditions, such as the nature of the acid, its concentration, the reaction temperature, and duration (*67*). If sufficient hydrolysis occurs then monosaccharides (sugars) will ultimately be produced; however further acid treatment results in the degradation /dehydration of the released monosaccharides producing 2-furfuraldehydes (from pentoses) and 5-hydroxymethylfurfural (from hexoses) (Figure 8). Furfural and HMF are the only furans usually found in hydrolysates in significant amounts (*66*, *68*). HMF further degrades to levulinic acid and formic acid (*10*).

It has also been reported that the dilute acid hydrolysis affects the substrate features such DP and crystallinity of the cellulose (*69*). Hydrolysis with dilute acid yields hydrocellulose, a product with reduced degree of polymerization (DP) but higher crystallinity (*70*).

Alkaline Hydrolysis and Peeling Reaction

Alkali pulping/pretreatment refers to the application of alkaline solutions to woody biomass to remove lignin that binds the fibers together, acetyl groups, and various uronic acid substitutions on hemicellulose that lower the accessibility of enzyme to the hemicellulose and cellulose (*71*). The mechanism of alkaline treatment is believed to include delignification, hydrolysis of polysaccharides, saponification of intermolecular ester bonds cross linking xylan hemicelluloses and other components, for example, lignin. The porosity of the lignocellulosic materials increases with the removal of the lignin and hemicellulose (*72*). Alkali treatment of lignocellulosic materials causes swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure (*73*).

The most important reactions responsible for the loss of polysaccharides and reduction of the chain length of cellulose are peeling and hydrolytic reactions. At elevated temperatures the polysaccharides are attacked by strong alkali solutions, with a large number of reactions taking place. The most important ones are: dissolution of undegraded polysaccharides, peeling of end-group, alkaline hydrolysis of glycosidic bonds and acetyl groups, degradation and decomposition

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of dissolved polysaccharides (*5*). In the aqueous environment during alkaline pulping, the reducing end group in the polysaccharide will form equilibrium between the hemiacetal and the open aldehyde form. In the presence of alkali, a further equilibrium will be established between the aldehyde and the keto form. This enediol is in equilibrium with the ketone but can also go through β-alkoxy elimination thus cleaving the glycosidic linkage (Figure 9).

Typical β-elimination occurs at the C-4 carbon atom, one hexose monomer unit is split off from the cellulose molecule, and the next glucose end group can take part in the reactions, resulting depolymerization known as Peeling-off reaction. Through another keto- enol tautomerization a ketone is formed. In the final step of the pathway, once the β-elimination has taken place, the liberated product can react further via a benzilic acid rearrangement to form a glucoisosaccharinic acid (*74*). The β-elimination can also occur at positions other than C-4; in that case the hexose unit remains attached to the cellulose molecule, which terminates the depolymerization, called the chemical stopping reaction (*75*).

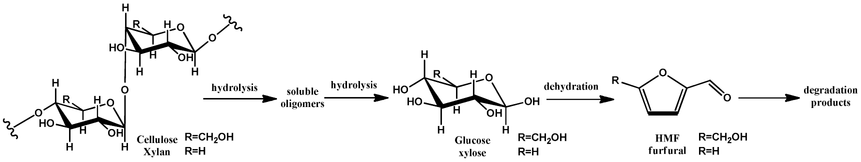
hydrolysis
soluble oligomers
hydrolysis
dehydration
degradation products
Cellulose R=CH₂OH
Xylan R=H
Glucose R=CH₂OH
xylose R=H
HMF R=CH₂OH
furfural R=H

Figure 8. Reaction scheme of cellulose and hemicellulose during acid hydrolysis (from ref. (67)).

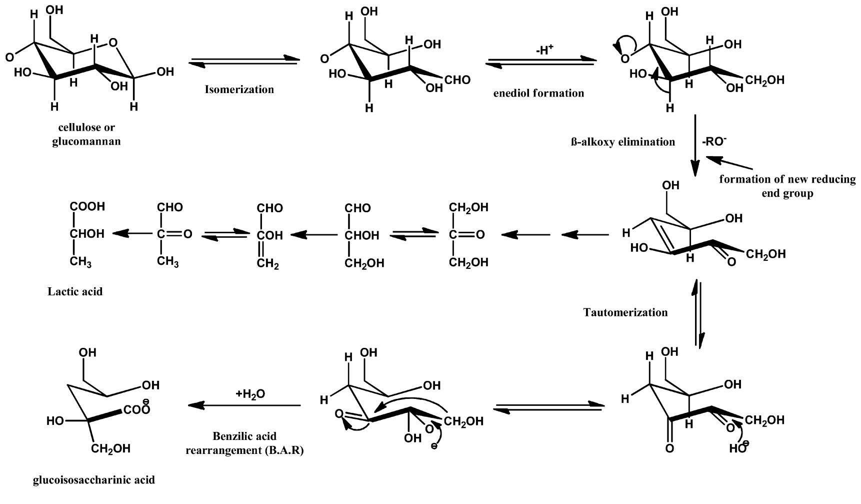
cellulose or glucomannan
Isomerization
-H⁺
enediol formation
ß-alkoxy elimination
-RO⁻
formation of new reducing end group
Tautomerization
COOH CHOH CH₃
Lactic acid
CHO C=O CH₃
CHO COH CH₂
CHO CHOH CH₂OH
CH₂OH C=O CH₂OH
+H₂O
Benzilic acid rearrangement (B.A.R)
glucoisosaccharinic acid

Figure 9. Peeling reaction pathway of cellulose during alkaline pulping (from ref. (74)).

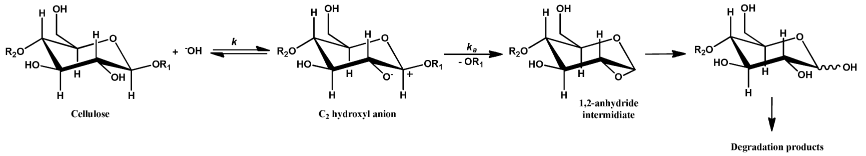

Figure 10. General reaction mechanism of alkaline hydrolysis of glycosidic linkages (from ref. (78)).

Figure 10 illustrates the generally accepted mechanisms for the alkali hydrolysis of glycosidic linkages (*76–78*). Alkali ionization of the C2-OH involves in the initial rapid equilibrium-controlled step. In the slow rate-limiting step, the C2 hydroxyl anion undergoes an intramolecular displacement process to yield a 1,2-anhydride intermediate (*78*).

Enzymatic Hydrolysis

The enzymatic hydrolysis of cellulose to glucose is generally accomplished by the synergistic action of three distinct classes of enzymes: i) the “endo-1,4-β-glucanases” or, 1,4-β-D-glucan 4-glucanohydrolases (EC 3.2.1.1), which act randomly on soluble and insoluble 1,4-β-glucan substrates and are commonly measured by detecting the reducing groups released from carboxymethylcellulose (CMC), ii) the “exo-1,4-β-D-glucanases”, including both the 1,4-β-D-glucan glucohydrolases (EC 3.2.1.74), which liberate D-glucose from 1,4-β-D-glucans and hydrolyze D-cellobiose slowly, and 1,4-β-D-glucan cellobiohydrolase (EC 3.2.1.91), which liberates D-cellobiose from 1,4-β-glucans, and iii) the “β-D-glucosidases” or β-D-glucoside glucohydrolases (EC 3.2.1.21), which act to release D-glucose units from cellobiose and soluble cellodextrins, as well as an array of glycosides (*45*, *68*). Besides these hydrolytic enzymes (endo- and exo-gluconases), the enzymes complexes active in the biodegradation of cellulose frequently also contain oxidatively acting enzyme systems, generally termed gluco-oxidases. They may play a part in generating sites enhancing hydrolytic enzyme attack, especially in well-ordered regions of the cellulosic substrate (*79*, *80*). Figure 11 illustrates the general reaction scheme of enzymatic hydrolysis of cellulose.

Because of xylan heterogeneity, the enzymatic hydrolysis of xylan requires different enzymatic activities. Two enzymes, β-1,4-endoxylanase (EC 3.2.1.8) and β-xylosidase (ED 3.2.1.37), are responsible for hydrolysis of the main chain (Figure 12), the former attacking the internal main-chain xylosidic linkages and the latter releasing xylosyl residues by means of endwise attack of xylooligosaccharides (*81*). An endoxylanase that cleaves β-(1,4) linkages will usually have no effect on β-(1,3) linkages.

In addition, an endoxylanase that cleaves main-chain linkages near an *O*-2-linked arabinose will have no effect on an open-chain xylan (*66*). However, for complete hydrolysis of hemicellulose, side chain cleaving enzyme activities are also necessary, such as, α-L-arabinofuranosidases (EC 3.2.1.55), endomannanases

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(EC 3.2.1.78), β-mannosidases (EC 3.2.1.25), and α-galactosidases (EC 3.2.1.22) (*82*). Hemicellulases are produced by a variety of fungus species; a combination of main- and side-chain enzymes is necessary to achieve complete hydrolysis of xylan and mannan (*66*).

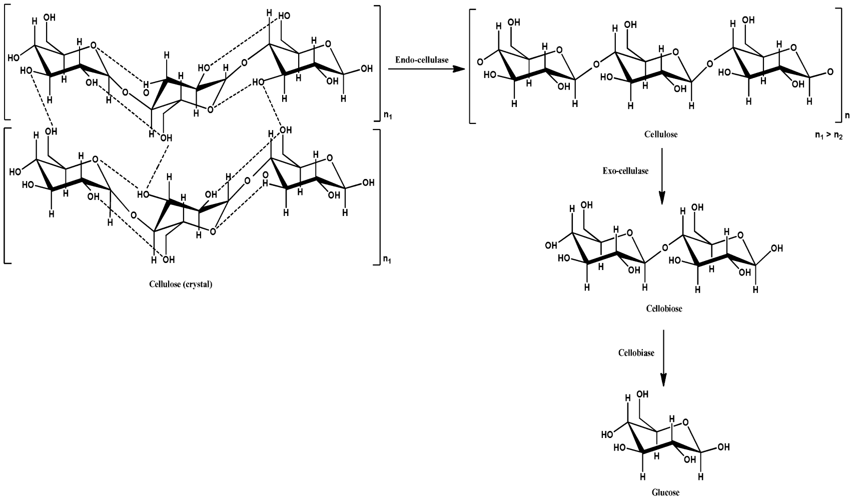
Endo-cellulase
Cellulose
n₁ > n₂
Exo-cellulase
Cellobiose
Cellobiase
Glucose
Cellulose (crystal)

Figure 11. Reaction scheme of enzymatic hydrolysis of cellulose (from ref. (45)).

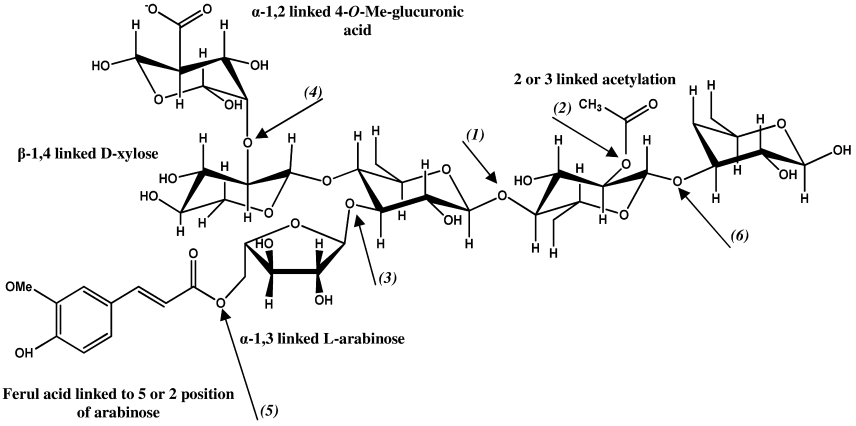
α-1,2 linked 4-O-Me-glucuronic acid
2 or 3 linked acetylation
β-1,4 linked D-xylose
α-1,3 linked L-arabinose
Ferul acid linked to 5 or 2 position of arabinose
(1)
(2)
(3)
(4)
(5)
(6)

Figure 12. Enzymatic reaction scheme of hemicellulose depolymerizing. Enzymes responsible: (1) endo-1,4-β xylanase, (2) acetyl xylan esterase, (3) α-L-arabinofuranosidase, (4) α-D-glucuronidase, (5) ferulic acid esterase, (6) β-xylosidase (from ref. (66)).

5.2. Reactions of Lignin

Reactions under Alkaline Conditions

The alkaline cleavage of α- and β-aryl ether linkages as well as condensation reactions have been extensively studied using lignin model compounds, but it is still not entirely clarified in the degradation of wood lignin. It is known that the dominant types of linkages found in both softwood and hardwood lignin are α-and β-aryl ether linkages, in particular the latter, the cleavage of these bonds contributes essentially to lignin degradation (*83–86*).

The main lignin-degradation reaction during alkaline pulping is the cleavage of β-*O*-4 linkages resulting in the formation of new phenolic hydroxyl groups. These lignin fragmentation reactions not only cause substantial decrease in the molecular mass of residual lignin, but also give it a more hydrophilic character by introducing new phenolic groups (*87*).

The phenolate anions formed upon protolysis of phenolic β-*O*-4 structures are converted into a quinone methide intermediate (Figure 13). Small portions of lignin are degraded by cleavage of less frequent linkages such as C-C bonds, leading to a reduction or total elimination of side-chains. For example, the γ-C may be split from quinone methide intermediates, releasing formaldehyde (*7*). The cleavage of β-*O*-4 linkages leading to the fragmentation of lignin then occurs through another nucleophilic attack by the thiol group at the α-carbon, or by another hydrosulfide ion during kraft pulping using Na_2S and NaOH.

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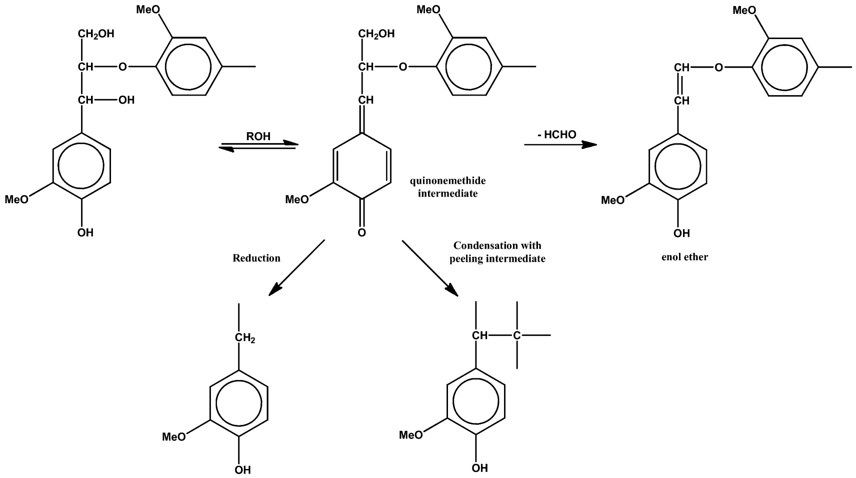
MeO
CH2OH
CH
O
CH
OH
MeO
OH
ROH
quinonemethide intermediate
O
- HCHO
enol ether
Reduction
Condensation with peeling intermediate
CH2
CH
C

Figure 13. Reaction scheme of alkaline cleavage of β-aryl ether linkage in phenolic lignin unit (from ref. (88)).

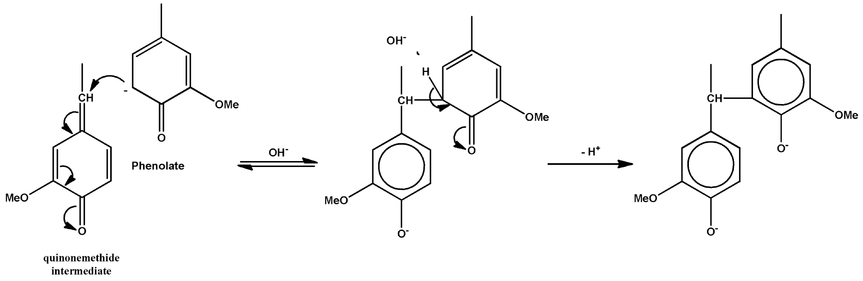

Figure 14. Primary condensation reaction of lignin in alkaline (from ref. (88)).

Condensation in alkaline medium, an undesirable reaction, can be generally described as an addition of internal nucleophiles (carbanions from phenolic or enolic structures) to conjugated carbonyl structures such as quinone methide or other enones. Gierer and Wannstrom (*89*) proposed that the condensation of quinone methide with polysaccharide peeling intermediate forming lignin carbohydrate complex (LCC) and could lead to restriction of dissolution of lignin since condensation reactions compete with reactions leading to dissolution of lignin and due to increased molecular weight.

Figure 14 illustrates the primary condensation reaction, if a phenolated unit is added to a quinone methide intermediate a new α-5 linkage is formed by an irreversible release of a proton and further to from a new C-C bond, a product of high molecular weight lignin (*7*, *88*).

Reactions under Acidic Conditions

In the dilute acid processing of lignocellulose, especially under high-temperature conditions, part of the lignin is solubilized into liquid due to partial cracking of lignin. A substantial fraction of this lignin (often referred to as acid-soluble lignin) actually precipitates out from the sugar solution during storage at room temperature (*7*). The precipitation is believed to be the result of recondensing of low molecular weight lignins. The acid cracking reaction of lignin is initiated by protonation of the benzyl oxygen followed by α-ether elimination to give a benzylic carbonium ion intermediate (Figure 15).

These hydrolytic reactions are often accompanied by condensations that would have a significant impact on reactivity of the resulting lignin. The reactions of phenyl units and reaction conditions have been shown to influence lignin condensation reaction. Lai (*78*) reported that syringyl nuclei condensed more readily than guaiacyl nuclei with vanillyl alcohol. Typical reactions involving carbonium ions can be visualized e.g. by the color transformation from yellow to green (a condensation product) when wood is treated with concentrated sulfuric acid (Figure 16). Treatments of wood with concentrated mineral acids do not cause dissolution but condensation reactions of lignin, a fact that is used in analytical lignin determination (klason lignin method).

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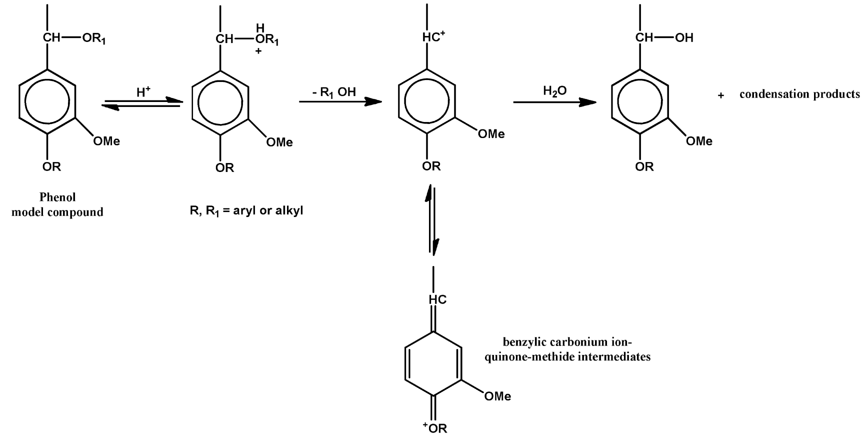
CH—OR1
OMe
OR
Phenol
model compound
H+
H
CH—OR1
+
OMe
OR
R, R1 = aryl or alkyl
- R1 OH
HC+
OMe
OR
H2O
CH—OH
OMe
OR
+ condensation products
HC
benzylic carbonium ion-
quinone-methide intermediates
OMe
+OR

Figure 15. Acid cleavage of α-ether linkages in lignin (from ref. (78)).

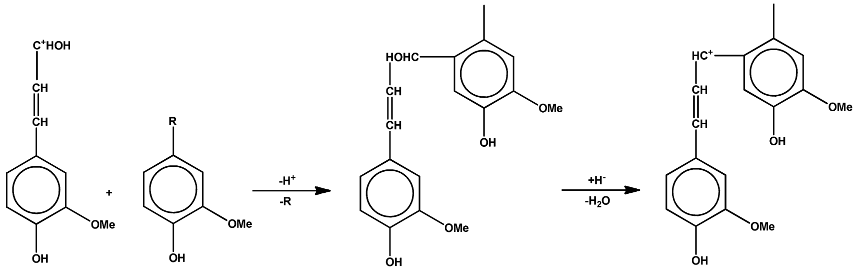
C+HOH
CH
CH
OMe
OH
+
R
OMe
OH
-H+
-R
HOHC
CH
CH
OMe
OH
OMe
OH
+H-
-H2O
HC+
CH
CH
OMe
OH
OMe
OH

Figure 16. General scheme of condensation reaction of lignin with concentrated acid (from ref. (7)).

Reactions in Organosolv Processes

Organosolv pulping/pretreatment process may be defined as the process of delignification of wood using organic solvents, and it has been the subject of considerable research activity in the past two decades (*90*, *91*). The two broad categories of organosolv processes are catalyzed and uncatalyzed. The catalysts can be acids, bases or salts. The ability of the organic/aqueous solvent to selectively extract high yields of the lignin fraction or a combination of lignin and hemicellulose fractions suggests that potentially these processes could be used as a pretreatment for the production of relatively high purity cellulosic substrates for enzyme hydrolysis (*92*, *93*). The most prevalent solvents used are methanol, ethanol, acetic acid, and formic acid. Other organic solvents are phenol, amines, glycols, nitrobenzene, dioxane, dimethylsuphoxide, sulfolene, and carbon dioxide (*94*).

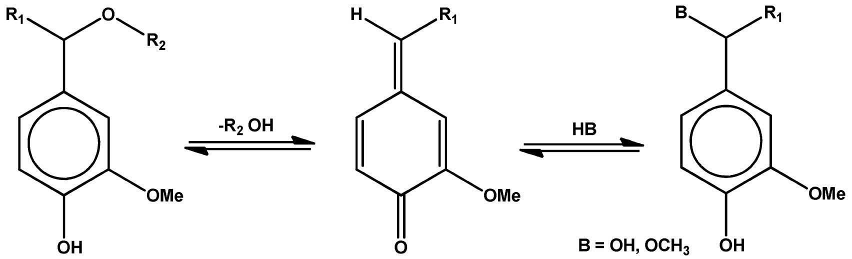

Figure 17. Scheme of solvolytic cleavage of a phenolic α-aryl ether (from ref. (95)).

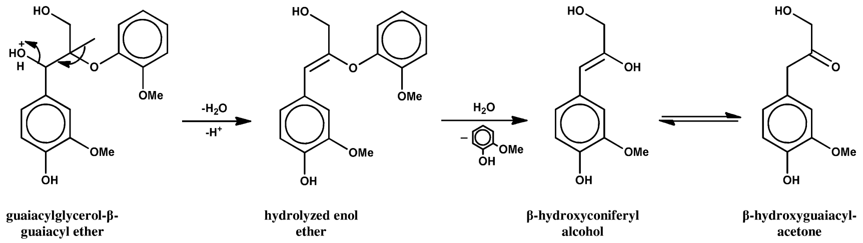
HO
HO
H
O
OMe
OMe
OH
-H2O
-H+
HO
O
OMe
OMe
OH
H2O
OMe
OH
HO
OH
OMe
OH
HO
O
OMe
OH
guaiacylglycerol-β-guaiacyl ether
hydrolyzed enol ether
β-hydroxyconiferyl alcohol
β-hydroxyguaiacyl-acetone

Figure 18. Scheme of solvolytic cleavage of β-aryl ether linkage (from ref. (95)).

Studies in the field of lignin chemistry concluded that the cleavage of ether linkages is primarily responsible for lignin breakdown in organosolv processes (*5*, *95*). The responsible reactions, which very likely occur in organosolv pulping, are believed to consist primarily of the solvolytic splitting of α-ether linkages. Model compound studies (*96*) have shown that α-aryl ether linkages are more easily split than β-aryl ether linkages, especially when they occur in a lignin structural unit containing a free phenolic hydroxyl group in the *p*-position. In this case the formation of a quinonemethide intermediate is possible (Figure 17).

Easily hydrolysable α-aryl ether bonds are most readily broken, but it is likely that β-aryl ether bonds are also broken under the conditions of many processes. The possibility of β-ether cleavage is greater in more strongly acidic systems. When spruce milled wood lignin is refluxed with 0.2M HCl in 9:1 dioxane-water, the products include several carbonyl compounds having the guaiacyl propane carbon skeleton. Figure 18 illustrates the cleavage of β-ether linkage when the model compound is refluxed with dioxane-water containing HCl (*97*). Similar compounds are obtained by Hibbert's group upon ethanolysis of spruce wood; these compounds are called Hibbert's ketones (*98*).

Inter- and intra-molecular condensation is also likely to play a role during organosolv pulping. Condensation at α-position of the side chain can be readily illustrated by reactions of lignin model compounds. Ede and Brunow (*99*) obtained high yields of the condensation product by reacting a veratrylglycerol-β-guaiacyl ether derivative with propylveratrole in refluxing 85% formic acid. It is noted that β-hydroxyconiferyl alcohol is unable to undergo β-ether cleavage because of the unavailability of an α-hydroxyl to serve as a leaving group (Figure 19), so its

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formation represents an irreversible condensation process. It is well established that hardwoods are easier to delignify with organic solvents than softwoods (*95*, *100*). Besides the differences in the total lignin content, the structural differences of lignins also play an important role. Hardwood lignins are richer in α-aryl ether linkages that are easily split in acid-organosolv pulping. Moreover, the higher amount of syringyl units inhibits condensation reactions (*95*).

Important parameters governing the course of delignification are pH, physical properties of the solvent (that govern its ability to dissolve lignin fragments), and chemical properties of the solvent (that govern its ability to participate in fragmentation reactions or inhibit recondensation of hydrolyzed lignin (*95*, *101*).

Reactions with Sulfite

The sulfite-based pulping and pretreatment process use sulfurous acid and sulfite for delignification (*102–105*). The process can be carried out over the wide pH range with different bases, sodium, magnesium, ammonium or calcium to buffer the cooking solution (*106*, *107*). Basically, two types of reactions, sulfonation and hydrolysis, are responsible for delignification in sulfite pulping. Sulfonation occurs at α-position and generates hydrophilic sulfonic acid groups in the hydrophobic lignin polymer, while hydrolysis breaks ether bonds between the phenylpropane units, lowering the molecular weight and creating free phenolic hydroxyl groups (*108*). Both of these reactions increase the hydrophilicity of the lignin, rendering it more soluble (*5*). Lignins produced by the sulfite pulping process are known as lignosulfonate, which has wide applications because of its features similar to surfactants (*109*).

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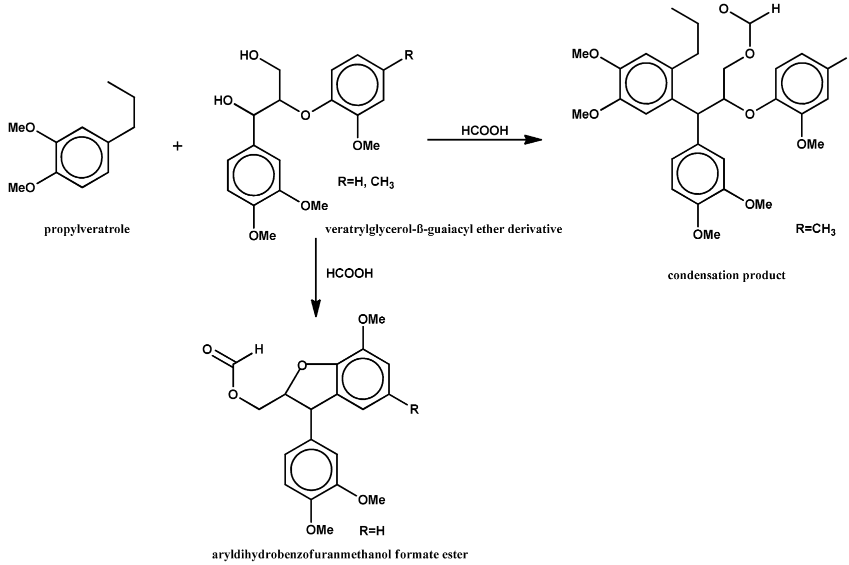

Figure 19. Scheme of inter-and intra-molecular condensations of lignin model compounds in formic acid (from ref. (95)).

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In general, sulfite pulping process can be broadly classified into three: neutral, acid, and alkaline sulfite pulping. In neutral sulfite pulping process the wood chips are cooked with Na_2SO_3 liquor buffered with either $NaHCO_3/Na_2CO_3$ or NaOH to maintain a slightly alkaline pH during the cook (*110*). The most important reactions of lignin are restricted to phenolic lignin units only in neutral sulfite pulping. The first stage always proceeds via the formation of a quinonemethide with simultaneous cleavage of an α-hydroxyl or an α-ether group. Acid sulfite pulping uses combination of sulfur dioxide solution containing different bases at high temperatures and pressures. The most active nucleophile present is the bisulfite ion, which forms via the following two equilibrium reactions (Eqns. 3 and 4) when sulfur dioxide is dissolved in water (*74*):

$$SO_2.H_2O + H_2O \leftrightarrow HSO_3^- + H_3O^+ \tag{3}$$

$$HSO_3^- + H_2O \leftrightarrow SO_3^{2-} + H_3O^+ \tag{4}$$

During acid sulfite pulping the α-hydroxyl and the α-ether groups are cleaved readily under simultaneous formation of benzylium ion, as illustrated in Figure 20. In principle, both phenolic and etherified lignin units can react, although the rates for phenolic structures seem to be somewhat higher (*83*, *111*). The bezylium ions are sulfonated by attack of hydrated sulfur dioxide or bisulfite ions present in the cooking liquor. The benzylium ions formed from the 1,2-diarylpropane structure are easily converted to stilbene structures by elimination of a hydrogen ion at the β-position.

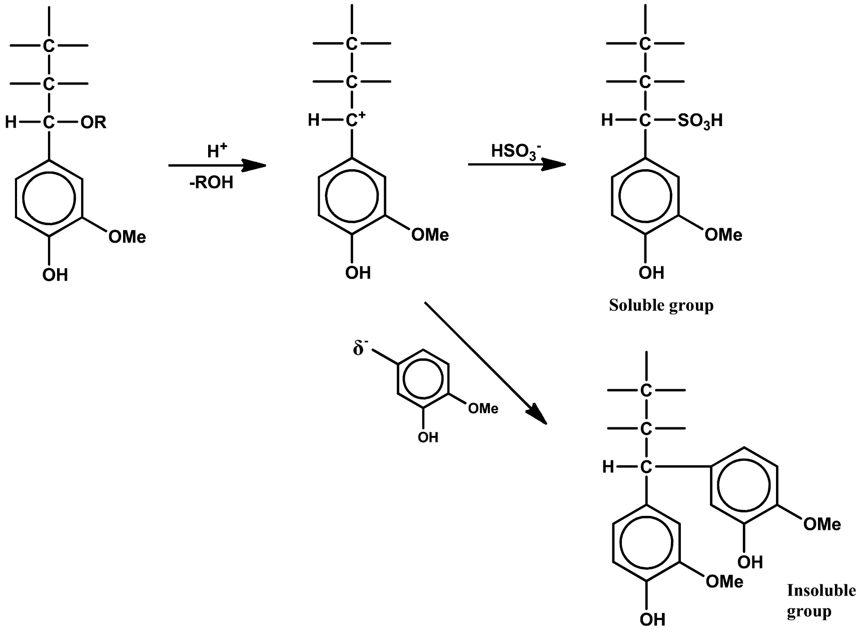
There is an undesirable acid catalyzed condensation reaction also occur during acid sulfite pulping (*112*). According to Sjostrom (*5*), condensation reactions of carbonium ions compete with sulfonation and their frequency is increased with increasing acidity. In general, the condensation reactions result in increased molecular weight of the lignosulfonates and the solubilization of lignin is inhibited. Although adequate information is lacking, the reactions of lignin with alkaline sulfite are largely related to those occurring in neutral sulfite and alkali pulping (*5*, *106*).

6. Influence of Substrate Characteristics on Enzymatic Saccharification of Cellulose

By nature, lignocellulosic materials are resistant to biodegradation. The root cause of biomass recalcitrance could be attributed to a number of factors, such as cellulose crystallinity, cellulose degree of polymerization (DP), substrate accessibility to cellulase, hemicellulose and lignin content, distribution, and structure (*113*).

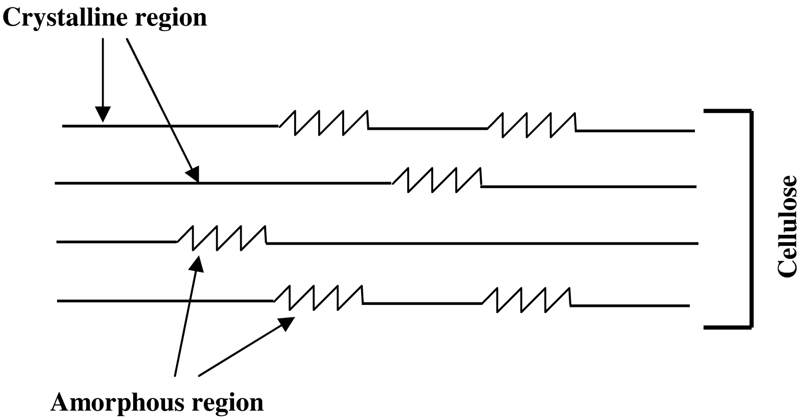
6.1. Crystallinity

The crystalline structure of cellulose is an important factor responsible for the lower digestibility of lignocellulosic biomass. The degree of crystallinity, which is a measure of the crystalline portion in a cellulose sample, depends on the origin of the cellulose. There is also a noncrystalline or amorphous portion in cellulose (Figure 21). The crystalline cellulose is more resistant to penetration by chemicals, enzymes, and water than the amorphous cellulose and is therefore more resistant to microbial degradation.



H—C—OR
OMe
OH
H⁺
-ROH
H—C⁺
HSO3⁻
H—C—SO3H
Soluble group
δ⁻
Insoluble group

Figure 20. Reaction mechanism of nucleophilic substitution of lignin during acid sulfite pulping (from ref. (112)).



Crystalline region
Cellulose
Amorphous region

Figure 21. Cellulose structure with crystalline and amorphous regions.

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The crystallinity of dried cellulose samples can be quantitatively measured from the wide range X-ray diffraction pattern. The crystallinity index (CrI) is one of the measures of crystalline degree, which can be calculated using equation (5) (*114*):

$$\text{Crystallinity Index } (\%) = \frac{\left(I_{002} - I_{am}\right)}{I_{am}} \times 100 \qquad (5)$$

Where I_{002} is the intensity of the peak at 22.8° and I_{am} is the intensity of peak at 18°. The values of crystallinity index for wood pulps range from 60% to 70%.

6.2. Degree of Polymerization

The number of glucose units in a cellulose molecular chain is referred to as the degree of polymerization (DP), which serves as a characteristic property of cellulose. The average DP for the molecular chains in a given sample is designated by $\overline{DP}$. Generally the degree of polymerization of cellulose ranges from less than 100 to several thousands. Goring and Timell (*115*) reported that wood cellulose has a $\overline{DP}$ of at least 9,000 to 10,000, and possibly as high as 15, 000 using nitration isolation procedure. A DP of 10, 000 would mean a linear chain length of approximately 5 μm (*28*, *116*). The DP of cellulose cannot be measured directly, but is calculated from intrinsic viscosity (also known as limiting viscosity number) of solutions prepared by dissolving cellulose in a suitable solvent, such as cupri-ethylenediamine (CED) or cadmium-ethylenediamine (cadoxen) (*117*), and it is expressed as the average molecular weight (M) divided by 162 (where 162 is the weight of an anhydro-glucose unit). It is accepted that an increase in DP would result in a network of fibers that are held together through hydrogen bonds forming long chains. In this form, the fibers are considered to be stronger, and comparatively resistant to solvents and degrading agents compared with those of low molecular weight. Structural features such as long chains and strong hydrogen bonding could limit the accessibility of cellulose to enzymes and thus diminish its susceptibility to hydrolysis (*118*). In the contrary, the reduction of DP could increase the number of cellulose chain ends available to the action of exoglucanase in the cellulase complex, thus generating high reaction rate and glucose yield (*119–122*).

6.3. Accessible Surface Area

Accessible surface area is widely recognized as a critical saccharification parameter due to the interfacial nature of cellulose hydrolysis (*73*). This factor is also referred to in the literature as sites accessible to enzyme, substrate accessibility to enzyme, and available pore volume. Stone et al. (*108*) hypothesized that the initial rate of hydrolysis is a function of cellulose accessible surface area. The tightly packed cellulose regions are a major factor in contributing to the resistance of cellulose to degradation, by limiting the accessibility to cellulases (*123*, *124*). It

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has been proposed that, to achieve efficient enzymatic saccharification, cellulose chains in the highly ordered and tightly packed regions of microfibrils should rather be delaminated, disrupted, thereby increasing the surface area and making the individual cellulose molecules more accessible and available for interactions with cellulose-degrading enzymes (*125–127*).

Solute exclusion technique is most commonly used to assess the substrates surface area (*128*). Although the solute exclusion method is common and effective, it requires a significant investment in time (*129*). A quantitative assay for determining accessible surface area has been established based on adsorption of a non-hydrolytic fusion protein (TGC) containing a cellulose-binding module and a green fluorescence protein (*130*). This approach more accurately assesses substrate characteristic related to enzymatic cellulose hydrolysis than traditional methods such as nitrogen adsorption-based Brunauer-Emmett-Teller (BET), size exclusion, and small angle X-ray scattering (*121*). More recently, rapid methods such as Simons Staining (SS) and water retention value measurements were proved to be good predictive methodologies for indicating the likely ease of enzyme accessibility and eventual hydrolysis effectiveness (*131*).

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6.4. Recalcitrance from Hemicellulose

Hemicelluloses are the second major sugar fraction after cellulose in lignocellulosic materials. However, their existence and linkage to lignin presents a major hurdle. It is believed that hemicellulose forms a physical barrier around the cellulose, which physically blocks cellulases from accessing cellulose (*132*, *133*). The hemicellulosic component of lignocellulosic biomass, xylan in particular, has been frequently cited as a physical barrier around cellulose within the lignocellulosic matrix, restricting access of cellulase to cellulose. As detailed in Section 4.2, xylans are heteropolysaccharides with homopolymeric backbone chains of 1,4-linked β-D-xylopyranose units (*134*). Hemicellulose removal increases the porosity of the native lignocellulosics and, thus, enzymatic accessibility to the cellulosic faction (*135*, *136*). In evidence, Bura et al. (*137*) reported that xylan content influenced both the enzyme requirements for hydrolysis and the recovery of sugars from the lignocellulosic materials. In the context of hemicellulose linkages, it is proposed that more linear hemicelluloses are tightly linked to cellulose and they are less water-soluble (*138*), while highly branched hemicelluloses are slightly linked to cellulose and they are more water-soluble (*5*). Thus, complete removal of the hemicellulose from the lignocellulosic material during pretreatment is a necessary prerequisite for the successful enzymatic hydrolysis of the cellulosic fraction.

6.5. Recalcitrance from Lignin

Woody biomasses contain approximately 20-30% lignin. Wood is similar to fiber-reinforced plastics, in which lignin represents the plastic binder and cellulose the reinforcing fiber (Figure 22). A lignified wood tissue is simply so compact that the polysaccharide degrading enzymes excreted by microorganisms cannot penetrate into the cell wall, and lignin serves as a barrier against the

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

cellulose hydrolytic enzymes. Studies demonstrated that removal of lignin could dramatically prompt enzymatic hydrolysis of cellulose. For example, steam exploded Douglas fir had poor digestibility because of very high lignin content (42%). When the lignin was removed by hydrogen peroxide (*139*) or oxygen-alkali (*140*), cellulose could be easily hydrolyzed completely.

In addition to the physical barrier, chemical bonding between lignin and carbohydrate seems another factor retarding enzymatic hydrolysis of cellulose. For example, in softwood galactomannan, arabino-4-*O*-methylglucouronoxylan, and arabinogalactan linked to lignin at benzyl positions (*141*, *142*), while in hardwood and grass lignin-carbohydrate complexes are composed exclusively of 4-*O*-methylglucuronoxylan and arabino-4-*O*-methylglucuronoxylan, respectively (*143*). Figure 23 illustrates the ester linkage occur between lignin and 4-*O*-methylglucuronoxylan in softwood (pine). The principle structural possibilities of lignin-polyoses complexes include: covalent and hydrogen bonds between one lignin element and one polysaccharide element, lignin elements bound to a polysaccharide element and polysaccharide elements bound to a lignin element, and finally a network of polysaccharide and lignin elements linked by chemical and physical linkages (*7*).

Lignin is also believed to irreversibly and nonproductively adsorb enzymes and thereby prevent them from working on cellulose (*144–146*). Physically blocking or covering lignin prior to enzyme loading could reduce lignin-enzyme interactions and enhance cellulose saccharification. Exogenous protein (for example, bovine serum albumin, BSA) has been used to saturate lignin for reducing adsorption of cellulase and enhancing enzymatic cellulose saccharification (*146*, *147*). Surfactants have also been used as lignin-blocking agents to improve enzymatic hydrolysis (*144–149*).

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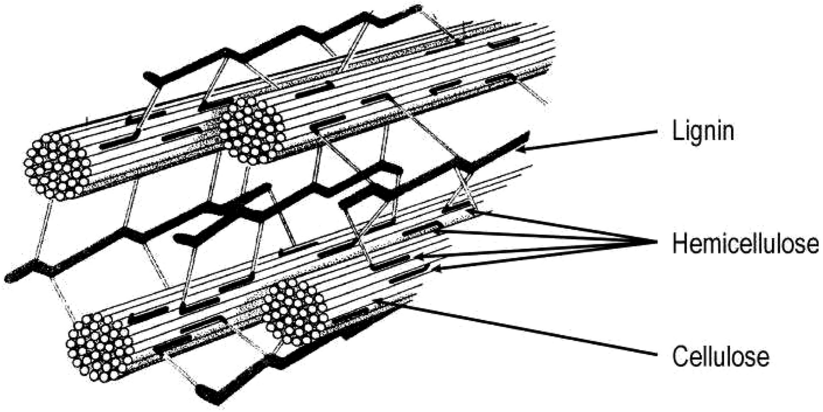

Figure 22. Schematic representation of lignin distribution in wood.

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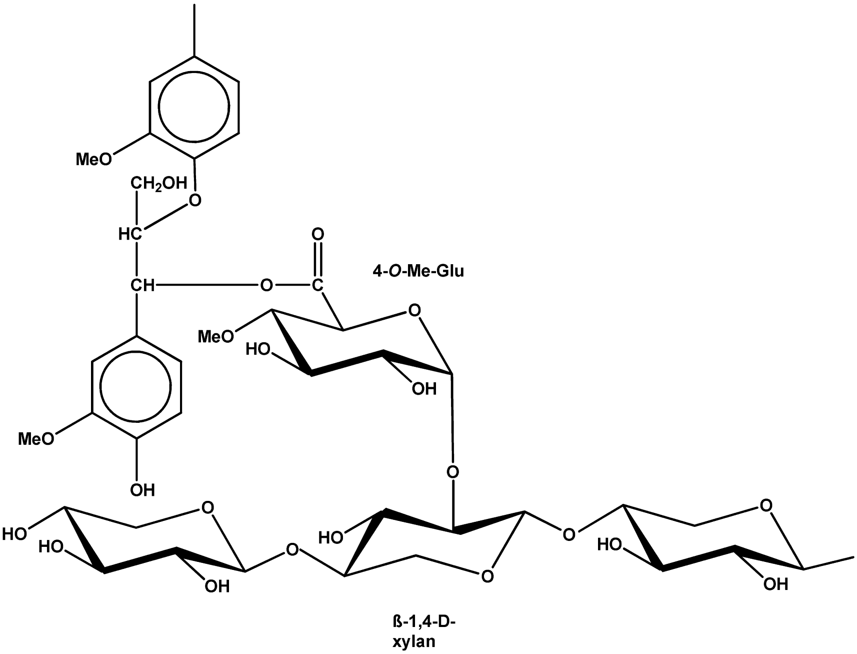

Figure 23. Proposed structure of ester linkage between lignin and 4-O-methylglucuronoxylan in softwood (from ref. (150)).

It has been shown that the influence of lignin on enzymatic hydrolysis of cellulose is related to lignin source and structure. For example, it is known that softwood lignin is more resistant than hardwood lignin to removal, and consequently to enzymatic hydrolysis (*125*). Another evidence is that selectively removing active fractions of lignin could reduce lignin inhibition to enzymes. For example, extracting steam exploded Douglas-fir with 1% NaOH at room temperature only reduced lignin content from 43% to 36%, but enzymatic hydrolysis of the substrate was enhanced by 30% (*147*). This suggested that the alkali-extractable lignin was more inhibitive to enzymes. Furthermore, functional groups in lignin play a role in lignin-enzyme interactions. It was reported that free phenolic hydroxyl groups are critical in inhibiting enzymes, and that selectively blocking (etherifying) the hydroxyl groups could reduce/remove the inhibition of the lignin (*122*).

References

1. Perlack, R. D.; Wright, L. L.; Turhollow, A.; Graham, R. L.; Stokes, B.; Erbach, D. C. *U. S. Dep. Ener. Agric. For. Serv.* **2005**, 73.
2. Galik, S.; Robert, Abt.; Wu, Y. *J. Forest.* **2009**, *107*, 69.
3. Hall, J. P. *For. Chron.* **2002**, 391.
4. Madgwick, H. A. I. In *Forest Biomass*; Madgwick, H. A. I., Ed.; Kluwer Academic Publishers: Netherlands, 1982; p 5.

5. Sjostrom, E. In *Wood Chemistry Fundamentals and Applications*; Sjostrom, E., Ed.; Academic Press: San Diego, 1993; p 2.
6. Haygreen, L.; Bowyer, J. L. In *Forest Products and Wood Science*; Haygreen, L., Bowyer, J. L., Eds.; Iowa State University Press: Iowa, 1989; p 101.
7. Fengel, D.; Wegener, G. In *Wood Chemistry, Ultrastructure, Reactions*; Fengel, D.; Wegener, G., Eds.; Walter de Gruyter: New York, 1984; p 7.
8. Lunnan, A.; Stupak, I.; Ansikainen, A.; Raulund-Rasmussen, K. In *Sustainable Use of Forest Biomass for Energy*; Roser, D., Asikainen, A., Raulund-Rasmussen, K., Stugpak, I., Eds.; Springer Publishers: Netherland, 2008; p 1.
9. Smith, B. W.; Miles, D. P.; Vissage, S. J.; Pugh, A. S. *U. S. Dep. Ener. Agric. For. Serv.* **2002**, 3.
10. Mousdale, M. D. In *Biofules: Biotechnology, Chemistry, and Sustainable Development*; Mousdale, M. D., Ed.; Taylor and Francis: Florida, 2008; p 41.
11. Gingras, J. *Biomass Bioenergy* **1995**, *9*, 153.
12. Stokes, B. J. *Biomass Bioenergy* **1992**, *2*, 131.
13. Kumar, P.; Barrett, D. M.; Delwiche, M. J.; Stroeve, P. *Ind. Eng. Chem. Res.* **2009**, *48*, 3713.
14. Pettersen, R. C. In *The Chemistry of Solid Wood*; Rowell, R. M., Ed.; Advances in Chemistry Series 207; American Chemical Society: Washington, DC, 1984; p 119.
15. Bowyer, L. J.; Shmulsky, R.; Haygreen, G. J. In *Forest Products and Wood Science: An Introduction*; Bowyer, L. J., Shmulsky, R., Haygreen, G. J., Eds.; Blackwell Publishing: Iowa, 2007; p 47.
16. Bidlack, J.; Malone, M.; Benson, R. *Proc. Okla. Acad. Sci.* **1992**, *72*, 51.
17. Timell, T. E. *Wood Sci. Technol.* **1967**, *1*, 45.
18. Brimacombe, J. S.; Ferrier, R. J.; Guthrie, R. D.; Hughes, N. A. In *Carbohydrate Chemistry*; Brimacombe, J. S., Ed.; The Chemical Society: Bristol, 1970; p 232.
19. Obst, J. R. *Holzforschung* **1982**, *36*, 143.
20. Morrell, J. J.; Gartner, B. L. In *Forest Products Biotechnology*; Bruce, A.; Palfreyman, W. J., Eds.; Taylor and Francis: London, 1998; p 1.
21. Panshin, A. J.; de Zeeuw, C. In *Textbook of Wood Technology*; Panshin, A. J., de Zeeuw, C., Eds.; McGraw-Hill: New York, 1970; p 15.
22. Tsoumis, G. T. In *Science and Technology of Wood: Structure, Properties, Utilization*; Tsoumis, G. T., Ed.; van Nostrand Reinhold: New York, 1991; p 32.
23. Savidge, R. A. In *Wood Quality and Its Biological Basis*; Barnett, J. R., Jeronimidis, G., Eds.; Blackwell Publishing, Ltd.: London, 2003; p 49.
24. Schiavone, A.; Guo, K.; Tassone, S.; Gasco, L.; Hernandez, E.; Denti, R.; Zoccarato, I. *Poultry Sci.* **2008**, *87*, 521.
25. Jackson, R. S. In *Wine Science: Principles, Practice, Perception*; Jackson, R. S., Ed.; Academic Press: California, 2000; p 258.
26. Kai, Y. In *Wood and Cellulosic Chemistry*; David, N.-S. H., Nobuo, S., Eds.; Marcel Dekker, Inc.: New York, 1991; p 215.

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27. Baeza, J.; Freer, J. In *Wood and Cellulose Chemistry*; Hon, D. N.-S., Shiraishi, N., Eds.; Marcel Dekker; Inc.: New York, 2001; p 275.
28. Ishii, T.; Shimizu, K. In *Wood and Cellulose Chemistry*; Hon, D. N.-S., Shiraishi, N., Eds.; Marcel Dekker Inc.: New York, 2001; p 175.
29. Dyas, L.; Goad, L. J. *Phytochem.* **1993**, *34*, 17.
30. Hafizoglu, H.; Sivrikaya, H.; Bacak, L. *Holz Roh. Werkst.* **2006**, *64*, 247.
31. Schulz, H.; Popp, P.; Huhn, G.; Stark, H. J.; Schuurmann, G. *Sci. Total Env.* **1999**, *232*, 49.
32. Wagenfuhr, V.; Scheiber, C. *VEB Fachbachverlaz. Leipzig* **1974**, *2*, 31.
33. Lee, H. N.; Stemphenson, J. N.; Hovey, R. W.; Turner, S. R.; Johnsen, B.; Larchar, A. B.; Thorsen, K. M.; Mason, J. O.; Kloss, T. E.; Hanson, H. H.; Clinc, M.; Buncke, H. J. In *The Manufacture of Pulp and Paper*; Lee, H. N., Ed.; McGraw-Hill Book Company: London, 1922; p 51.
34. Young, R. A. In *Kent and Riegel's Handbook of Industrial Chemistry and Biotechnology*; Riegel, R. E., Kent., Eds.; Springer Science: New York; 2007; p 1234.
35. Kollmann, F.; Fengel, D. *Holz Roh. Werkst.* **1965**, *23*, 461.
36. Browning, B. l.; Isenberg, I. H. In *Wood Chemistry*; Wise, L. E., Jahn, E. C., Eds.; Reinhold Publications Corp.: New York, 1952; p 1259.
37. Timell, T. E. *Wood Sci. Technol.* **1982**, *16*, 83.
38. Savard, J.; Nicolle, J.; Andre, A. M. *Analyse chimique des bios tropicaux.* **1960**, *3*, 5.
39. Markovic, N.; Terzan, N.; Pjevic, V. *Sumarstvo.* **1966**, *19*, 17.
40. Buchanan, M. A. In *The Chemistry of Wood*; Browning, B. L., Huntington, N. Y., Eds.; Robert E. Krieger Publishing Company: Florida, 1975; p 314.
41. Saka, S. In *Wood and Cellulose Chemistry*; Hon, D. N.-S., Shiraishi, N., Eds.; Marcel Dekker, Inc.: New York, 2001; p 51.
42. Rowell, R. M.; Pettersen, R.; Han, J. S.; Rowell, J. S.; Tshabalala, M. A. In *Handbook of Wood Chemistry and Wood Composites*; Rowell, R. M., Ed.; CRC Press: Finland, 2005; p 35.
43. Anderson, E. *J. Biol. Chem.* **1936**, *112*, 531.
44. Jahnel, F. *Manual Guit. Technol.* **2000**, *1*, 60.
45. Olsson, L.; Jorgensen, H.; Krogh, K. B. R.; Roca, C. In *Polysaccharides: Structural Diversity and Functional Versatility*; Dumitriu, S., Ed.; Marcel Dekker: New York, 2005; p 957.
46. VanderHart, D. L.; Atalla, R. H. In *Cellulose: Structure, Modification and Hydrolysis*; Young, R. A., Rowell, R. M., Eds.; Wiley Interscience: New York, 1986; p 88.
47. Perez, S.; Mazeau, K. In *Polysaccharides: Structural Diversity and Functional Versatility*; Dumitriu, S., Ed.; Marcel Dekker: New York, 2005; p 41.
48. Kondo, T. In *Polysaccharides: Structural Diversity and Functional Versatility*; Dumitriu, S., Ed.; Marcel Dekker: New York, 2005; p 69.
49. Bulian, F.; Graystone, J.; In *Wood Coatings: Theory and Practice*. Bulian, F., Graystone, J., Eds.; Elsevier: Amsterdam, 2009; p 19.
50. Jones, R. W.; Krull, J. H.; Blessin, C. W.; Inglett, G. E. *Cereal Chem.* **1979**, *56*, 441.

Downloaded by DUKE UNIV on June 16, 2012 | http://pubs.acs.org
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Downloaded by DUKE UNIV on June 16, 2012 | http://pubs.acs.org
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51. Hoegger, P.; Majchercyzk, A.; Dwivedi, R. C.; Svobodova, K.; Kilaru, S.; Kues, U. In *Wood Production, Wood Technology, and Biotechnological Impacts*; Kues, U., Ed.; Universitatsverlag Gottingen: Denmark, 2007; p 383.
52. Teeri, T.; Reinikainen, T.; Ruohonen, L.; Jones, T. A.; Knowles, J. K. C. *J. Biotechnol.* **1992**, *24*, 169.
53. Timell, T. E. *Adv. Carbohydr. Chem.* **1964**, *19*, 247.
54. Timell, T. E.; Syracuse, N. Y. *Wood Sci. Technol.* **1967**, *1*, 45.
55. Sarkanen, K. V.; Ludwig, C. H. In *Lignin: Occurrence, Formation, Structure and Reactions*. Sarkanen, K. V., Ludwig, C. H., Eds.; Wiley-Interscience: New York, 1971; p 916.
56. Alder, E. *Wood Sci. Technol.* **1977**, *11*, 169.
57. Chen, C. L. In *Methods in Lignin Chemistry*; Lin, S. Y., Dence, C. W., Eds.; Springer-Verlag: Berlin, 1992; p 409.
58. Gakkai, N. M. *J. Japan Wood Res. Soc.* **1985**, *31*, 363.
59. Henriksson, G. Lignin. In *Wood Chemistry and Wood Biotechnology*; Monica, Ek., Gellerstedt, G., Henriksson, G., Eds.; Walter de Gruyter GmbH Publishing Company: Berlin, 2009; p 121.
60. Lu, F.; Ralph, J. *J. Wood Chem. Technol.* **1998**, *18*, 219.
61. Demirbas, A.; Kucuk, M. M. *Cell. Chem. Technol.* **1993**, *27*, 679.
62. Harris, E. E. *Ind. Eng. Chem.* **1945**, *37*, 12.
63. Rashed, M. M.; EI-Sayed, S. T.; Hashem, A. M. *Bull. Fac. Pharm.* **1993**, *31*, 165.
64. Springer, E. L. *Tappi J.* **1966**, *49*, 102.
65. Lee, Y. Y.; Iyer, P.; Torget, R. W. In *Recent Progress in Bioconverion of Lignocellulosics*; Tsao, G. T., Ed.; Advances in Biochemical Engineering/Biotechnology; Springer-Verlag: Berlin, 1999; p 93.
66. Wyman, C. E.; Decker, R. S.; Himmel, E. M.; Brady, W. J.; Skopec, C.; Viikari, L. In *Polysaccharides: Structural Diversity and Functional Versatility*; Dumitriu, S., Ed.; Marcel Dekker: New York, 2005; p 995.
67. Theander, O.; Nelson, D. In *Advances in Carbohydrate Chemistry and Biochemistry*; Tipson, S. R., Horton, D., Eds.; Academic Press; Inc.: California, 1988; p 273.
68. Taherzadeh, M. J.; Karimi, K. In *Biofuels Refining and Performance*; Nag, A., Ed.; McGraw Hill Co.: New York, 2008; p 69.
69. Carrasco, J. E.; Saiz, Ma. C.; Navarro, A.; Soriano, P.; Saez, F.; Marinez, J. M. *Appl. Biochem. Biotechnol.* **1994**, *45/46*, 23.
70. Nelson, M. L. *J. Polym. Sci.* **1960**, *43*, 351.
71. Chang, V. S.; Holtzapple, M. T. *Appl. Biochem. Biotechnol.* **2000**, *22*, 177.
72. Tarkow, H.; Feist, W. C. *Chem. Ser.* **1969**, *95*, 219.
73. Fan, L. T.; Lee, Y. H.; Beardmore, D. H. *Biotechnol. Bioeng.* **1980**, *22*, 177.
74. Gellerstedt, G. In *Wood Chemistry and Wood Biotechnology*; Monica, Ek., Gellerstedt, G., Henriksson, G., Eds.; Walter de Gruyter GmbH Publishing Company: Berlin, 2009; p 173.
75. Pavasars, I.; Hagberg, J.; Boren, H.; Allard, B. *J. Poly. Env.* **2003**, *11*, 39.
76. Capon, B. *Chem. Rev.* **1969**, *69*, 407.

77. Overend, W. G. In *The Carbohydrates*; Pigman, W., Horton, D., Eds.; Academic Press: New York, 1972; p 279.
78. Lai, Y.-Z. In *Wood and Cellulose Chemistry*; Hon, D. N.-S., Shiraishi, N., Eds.; Marcel Dekker Inc.: New York, 1991; p 455.
79. Krassig, H,A. In *Cellulose: Structure, Accessibility, and Reactivity*; Krassig, H,A., Ed.; Gordon and Breach Science Publishers: Netherlands, 1996; p 175.
80. Wu, H.; Prei, J.; Wu, G.; Shao, W. *Enzyme Microb. Technol.* **2008**, *42*, 230.
81. Beg, Q. K.; Kapoor, M.; Mahajan, L.; Hoondal, G. S. *Appl. Microbiol. Biotechnol.* **2001**, *56*, 326.
82. Eriksson, T.; Borjesson, J.; Tjerneld, F. *Enzym. Microb. Technol.* **2002**, *3*, 353.
83. Rydholm, S. A. In *Pulping Processes*; Rydholm, S. A., Ed.; Interscience Publishers: New York, 1965; p 95.
84. Gierer, J. *Inter. Symp. Wood Pul.* **1981**, *2*, 12.
85. Gierer, J. *Holzforschung* **1982**, *36*, 43.
86. Gierer, J.; Noren, I. *Holzforschung* **1980**, *34*, 197.
87. Gellerstedt, G.; Rober, D. *Acta Chem. Scand.* **1987**, *41*, 541.
88. Gierer, J. *Wood Sci. Technol.* **1980**, *14*, 241.
89. Gierer, J.; Wannstrom, S. *Hofzforschung* **1986**, *40*, 347.
90. Lora, J. H.; Aziz, S. *Tappi J.* **1985**, *68*, 94.
91. Xu, Y.; Li, K.; Zhang, M. *Colloids Surf., A* **2007**, *301*, 255.
92. Pan, X. J.; Arato, C.; Gilkes, N.; Gregg, D.; Mabee, W.; Pye, K.; Xiao, Z.; Zhang, X.; Saddler, J. *Biotechnol. Bioeng.* **2005**, *90*, 473.
93. Pan, X. J.; Gilkes, N.; Kadla, J.; Pye, K.; Saka, S.; Gregg, D.; Ehara, K.; Xie, D.; Lam, D.; Saddler, J. *Biotechnol. Bioeng.* **2006**, *94*, 851.
94. Hergert, H. L. In *Tappy 1992 Solvent Pulping Symposium Notes*; Hergert, H. L., Ed.; Tappy Press: Atlanta, 1992; p 1.
95. McDonough, T. J. *Tappi J.* **1993**, *76*, 186.
96. Sakakibara, A.; Takeyama, H.; Morohoshi, N. *Holzforschung* **1966**, *20*, 45.
97. Adler, E.; Pepper, J. M.; Eriksoo, E. *Ind. Eng. Chem.* **1957**, *49*, 1391.
98. West, E.; MacInnes, A. S.; Hibbert, H. *J. Am. Chem. Soc.* **1943**, *65*, 1187.
99. Ede, R.; Brunow, G.; Poppius, K.; Sundquist, J.; Hortling, B. *Nordic Pulp Paper Res. J.* **1988**, *3*, 3.
100. Sarkanen, K. V. In *Progress in Biomass Conversion*; Sarkanen, K. V., Tillman, D. A., Eds.; Academic Press, New York, 1980; p 127.
101. Sakakibara, A.; Nakayama, N. *J. Japan Wood Res. Soc.* **1962**, *8*, 157.
102. Walker, J. C. F. In *Primary Wood Processing: Principles and Practice*; Walker, J. C. F., Ed.; Springer Publishers: Netherlands, 2006; p 477.
103. Wang, G.; Pan, X. J.; Zhu, J. Y.; Gleisner, R.; Rockwood, D. *Biotechnol. Prog.* **2009**, *25*, 1086.
104. Zhu, J. Y.; Pan, X. J.; Wang, G. S.; Gleisner, R. *Biores. Technol.* **2009**, *100*, 2411.
105. Shuai, L.; Yang, Q.; Zhu, J. Y.; Lu, F. C.; Weimer, P. J.; Ralph, J.; Pan, X. J. *Biores. Technol.* **2010**, *101*, 3106.
106. Grace, T. M.; Leopold, B.; Malcolm, E. W. In *Pulp and Paper Manufacture*; Kocurek, M. J., Ed.; TAPPI/CPPA Publishers: Atlanta, 1989; p 159.
107. Gierer, J. *Svensk Papperstidn.* **1970**, *73*, 571.

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108. Stone, J. E.; Scallan, A. M.; Donefer, E.; Ahlgren, E. *Adv. Chem. Ser.* **1969**, *95*, 219.
109. Yanhua, J.; Weihong, Q.; Zongshi, Li.; Lubai, C. *Energy Resour.* **2004**, *26*, 409.
110. Young, L. Y.; Frazer, A. C. *Geomicrobiol. J.* **1987**, *5*, 261.
111. Casey, J. P. In *Pulp and Paper Chemistry and Chemical Technology*; Casey, J. P. Wiley Interscience Publisher: New York, 1980; p 69.
112. Gellerstedt, G. *Svensk Papperstidn.* **1976**, *79*, 537.
113. Chandra, R. P.; Bura, R.; Mabee, W. E.; Berlin, A.; Pan, X.; Saddler, J. N. *Adv. Biochem. Eng. Biotechnol.* **2007**, *108*, 67.
114. Segal, L.; Creely, J. J.; Martin, Jr., A. E.; Conrad, C. M. *Text. Res. J.* **1959**, *29*, 786.
115. Goring, D. A.; Timell, T. E. *Tappi* **1962**, *45*, 454.
116. Lewin, M. In *Handbook of Fiber Chemistry*; Lewin, M., Ed.; Taylor and Francis: Finland, 2007; p 488.
117. Kaminska, E. *Mater. Res. Soc. Symp. Proc.* **1997**, *462*, 45.
118. Pan, X. J.; Xie, D.; Kang, K. Y.; Yoon, S. L.; Saddler, J. N. *Appl. Biochem. Biotechnol.* **2007**, *137*, 367.
119. Martinez, J. M.; Reguant, J.; Montero, M. A.; Montane, D.; Salvado, J.; Farriol, X. *Ind. Eng. Chem. Res.* **1997**, *36*, 688.
120. Valjamae, P.; Pettersson, G.; Johansson, G. *Eur. J. Biochem.* **2001**, *268*, 4520.
121. Zhang, Y. H.-P.; Lynd, L. R. *Biotechnol. Bioeng.* **2004**, *88*, 797.
122. Pan, X. J. *J. Biobased Mater. Bioenergy* **2008**, *2*, 25.
123. Wood, T. M.; McCrae, S. I.; Bhat, K. M. *Biochem. J.* **1989**, *260*, 37.
124. Laureano-Perez, L.; Teymouri, F.; Alizadeh, H.; Dale, B. E. *Appl. Biochem. Biotechnol.* **2005**, *121*, 1081.
125. Mansfield, S. H.; Mooney, C.; Saddler, J. N. *Biotechnol. Prog.* **1999**, *15*, 804.
126. Coughlan, M. P. *Biotechnol. Gene. Eng. Review.* **1985**, *3*, 37.
127. Arantes, V.; Saddler, J. N. *Biotechnol. Biofuels* **2010**, *3*, 4.
128. Mooney, C. A.; Mansfield, S. D.; Touhy, M. G.; Saddler, J. N. *Biores. Technol.* **1998**, *64*, 113.
129. Converse, A. O. In *Bioconversion of Forest and Agricultural Plant Residues*; Saddler, J. N., Ed.; CAB International Wollingford Publishers: Oxfordshire, 1993; p 93.
130. Hong, J.; Ye, X. H.; Zhang, Y. H. P. *Langmuir* **2007**, *23*, 12535.
131. Chandra, R. P.; Ewanick, S. M.; Chung, P. A.; Au-Yeung, K.; Rio, L. D.; Mabee, W.; Saddler, J. N. *Biotechnol. Lett.* **2009**, *31*, 1217.
132. Hsu, T. A. In *Handbook on Bioethanol: Production and Utilization*; Wyman, C. E., Ed.; Taylor and Francis: Washington, DC, 1997; p 179.
133. Yang, B.; Wyman, C. E. *Biotechnol. Bioeng.* **2004**, *86*, 88.
134. Brunecky, R.; Vinzant, T. B.; Porter, S. E.; Donohoe, B. S.; Johnson, D. K.; Himmel, M. E. *Biotechnol. Bioeng.* **2009**, *102*, 1537.
135. Kabel, M. A.; Bos, G.; Zeevalking, J.; Voragen, A. G. J.; Schols, H. A. *Biores. Technol.* **2007**, *98*, 2034.

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136. Laxman, R. S.; Lachke, A. H. In *Handbook of Plant-Based Biofuels*; Pandey, A., Ed.; CRC Press, Taylor and Francis Group: Finland, 2009; p 121.
137. Bura, R.; Chandra, R; Saddler, J. N. *Biotechnol. Prog.* **2009**, *25*, 315.
138. Lawther, J. M.; Sun, R. C.; Banks, W. B. *J. Agri. Food Chem.* **1995**, *43*, 667.
139. Yang, B.; Boussaid, A.; Mansfield, S. D.; Gregg, D. J.; Saddler, J. N. *Biotechnol. Bioeng.* **2002**, *77*, 678.
140. Pan, X. J.; Zhang, X.; Gregg, D. J.; Saddler, J. N. *Appl. Biochem. Biotechnol.* **2004**, *115*, 1103.
141. Azuma, J. I.; Takahashi, N.; Koshijima, T. *Carbohyd. Res.* **1981**, *93*, 91.
142. Mukoyoshi, S. I.; Azuma, J. I.; Koshijima, T. *Holzforschung* **1981**, *35*, 233.
143. Azuma, J. I.; Koshijima, T. *Methods Enzymol.* **1988**, *161*, 12.
144. Eriksson, T.; Borjesson, J.; Tjerneld, F. *Enzyme Microb. Technol.* **2002**, *31*, 353.
145. Mansfield, S. D.; Mooney, C.; Saddler, J. N. *Biotechnol. Prog.* **1999**, *15*, 804.
146. Yang, B.; Wyman, C. E. *Biotechnol. Bioeng.* **2006**, *94*, 611.
147. Pan, X. J.; Xie, D.; Gilkes, N.; Gregg, D. J.; Saddler, J. N. *Appl. Biotechem. Biotechnol.* **2005**, *124*, 1069.
148. Borjesson, J.; Peterson, R.; Tjerneld, F. *Enzym. Microb. Technol.* **2007**, *40*, 754.
149. Tu, M.; Zhang, X.; Paice, M.; McFarlane, P.; Saddler, J. N. *Biotechnol. Prog.* **2009**, *25*, 1122.
150. Watanabe, T.; Kushijima, T. *Agric. Biol. Chem.* **1988**, *52*, 2953.

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Chapter 6

The Influence of Lignin on the Enzymatic Hydrolysis of Pretreated Biomass Substrates

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The bioconversion of lignocellulosic biomass to ethanol has been proposed as a potential process to provide a more sustainable transportation fuel substitute for fossil fuels such as petrol and gasoline. However, the recalcitrance of lignocellulosic substrates remains a major challenge for the pretreatment and enzymatic hydrolysis steps of the overall biomass-to-ethanol process. Lignin, one of the major components of lignocellulosic biomass presents both chemical and physical barriers to the enzymatic hydrolysis of pretreated substrates. The two primary mechanisms include, lignins role in limiting access of the enzymes to the cellulose by its physical/mechanical role and location within the substrate and through the non-productive binding of cellulases to the lignin. Although hydrophobic, electrostatic, and hydrogen bonding interactions have been implicated in these cellulase-lignin interactions, the exact nature of this interaction remains to be more fully elucidated. To try to limit the non-productive binding of cellulases to lignin we and other groups have tried to determine the main factors that influence cellulase-lignin interaction. In the work described in this review we show how targeted changes to the chemical and physical properties of lignin, though varying both the pretreatment and biomass feedstock, can decrease the non-productive binding of cellulases to lignin.

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

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Introduction

The development of biorefineries that are capable of producing fuels and commodity chemicals from lignocellulosic biomass is viewed as a potential alternative to the world's current reliance on fossil fuels (*1*). Driving forces, including potentially higher fossil fuel prices and their contribution to CO_2 emissions, have catalyzed many groups to develop cost effective processes to produce biofuels such as ethanol from lignocellulosic biomass (*2*). The "typical" biomass-to-ethanol process usually consists of three main process steps including pretreatment, which involves the physical, mechanical or chemical breakdown of the cell wall structure to improve recovery of the hemicellulose and lignin components in a useful form as well as enhancing access of the cellulose to the cellulase complex. The other two process steps are enzymatic hydrolysis of the cellulose and the fermentation of the cellulose and hemicellulose derived sugars to ethanol. Several technical and economic issues have slowed the commercialization of the various biomass-to-ethanol processes (*3*). It is apparent that many of the technical challenges stem from the inherent recalcitrance of the biomass itself. Although cellulose, hemicellulose, and lignin are the primary structural components of most lignocellulosic materials, there are considerable differences between grasses, hardwoods and softwoods in terms of their content, composition and structure (*4*). Cellulose and hemicelluloses, which comprise about 65-75% of most biomass, substrates, can be broken down to their component sugars for fermentation to ethanol (*1a,5*). Lignin is generally composed of polymers of phenylpropane units, guaiacyl (G, coniferyl alcohol), syringyl (S, sinapyl alcohol), and *p*-Hydroxypheny units (H, *p*-coumaryl alcohol). The monolignols have been shown to be biosynthesized from glucose via phenylalanine (*4a*). The lignin composition varies depending upon the biomass source and type. For example, for the softwood *Pinus sylvestris*, the lignin's H and G content are 2% and 98% (*6*), for the hardwood *Populus euramericana*, the lignin's G and S content are 39% and 61%, and for corn stalks, the H, G, and S content of the lignin are 4%, 35%, and 61%, respectively. In addition, the cell walls in corn stover contain up to 4% ferulate and up to 3% *p*-coumarate (*7*). The lignin content also varies depending on the source of the lignocellulosic materials. Softwoods generally contain about 26-32% lignin, hardwoods contain 20-25% lignin (*4a*), while an agricultural residue such as corn stover generally has a lower lignin content of about 16-22%, when compared to wood derived biomass (*4a, 5a, 8*). The bond types of the various lignins subunits, also vary depending on the source of the lignocellulosic material (*4a*).

Although in some situations, the residual hemicellulose within the pretreated biomass substrate can significantly limit the ease of enzymatic hydrolysis of the cellulose (*8*, *9*), it is primarily the lignin and the various roles it plays in limiting access to the cellulose and in binding with the cellulase enzymes that have proven to be the main impediments to achieving effective enzymatic hydrolysis (*10*). For example, the removal of lignin has also been shown to facilitate the complete hydrolysis of the cellulose component regardless of the presence or absence of hemicellulose (*11*). As mentioned earlier, an effective pretreatment is one which both provides the ready fractionation and recovery of the hemicelluloses and lignin components in a useful form while enhancing access of the cellulose to the cellulase complex (*2a, 12*). An effective pretreatment should also be economical and robust enough such that it can be applied to a range of biomass substrates. Although an ideal pretreatment process would enhance the ready and complete separation and isolation of each component of the lignocellulosic substrate, this is likely to be impractical for both technical and economic reasons. As a result, pretreated substrates will typically contain at least some amount of hemicellulose and a lot more residual lignin associated with the cellulose rich, water or solvent insoluble stream obtained after pretreatment. The amount of each component remaining associated with the cellulosic fraction will vary depending on the type and severity of the pretreatment (*8*, *13*).

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Pretreatment and Enzymatic Hydrolysis of Lignocellulosic Biomass

Several pretreatments have been advocated over the years, including steam/dilute acid pretreatment (*14*), organosolv (*15*), ammonia fiber expansion/explosion (AFEX) (*16*), lime (*17*), dilute acid (*18*) and SPORL (*33*) pretreatments. However, each of the pretreatment processes has its own particular drawbacks and advantages (*2a, 16, 19*). As mentioned earlier, the lignin content of the residual cellulosic substrate will vary, depending on the pretreatment method used (*20*). In general, solvent based or alkaline pretreatments result in a more targeted removal of the lignin component, whereas, acidic pretreatments primarily solubilise the hemicelluloses leaving a cellulosic fraction with a high lignin content. For example, earlier comparative pretreatment studies showed that only a small amount of the lignin present in corn stover could be removed by dilute acid, whereas substantial amounts of lignin were removed by lime pretreatment (*21*). In other cases, the composition of the substrate after AFEX pretreatment was essentially the same as that of the original biomass (*21*), while the lignin content of steam pretreated substrates increased when compared to the initial substrates, mainly because of the solubilization and removal of the hemicellulose (*13*). Other work has shown that organosolv pretreatment decreased the lignin content in pretreated substrates due to the solubilization of the lignin into the organic solvent (*22*).

In addition to affecting the amount of lignin in the substrate, the choice of pretreatment will also affect the physical and chemical structure of lignin, altering both the accessibility to the cellulose and the interaction of cellulases with the pretreated substrate. There has been little work done on the effects of pretreatments on lignin structure and even fewer studies on the influence that lignin might play in binding to cellulases. However, some general trends can be anticipated. For example, it is likely that acidic pretreatments such as steam and dilute acid treatment would result in a net increase in condensed lignin, although simultaneous lignin depolymerization and condensation reactions are known to occur (*23*). It has also been suggested that "external lignin" that is often observed as droplets or re-precipitates on the surfaces of dilute acid, hydrothermal and steam pretreated substrates act as a physical barrier to cellulases

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(*24*) . Although the exact mechanisms have not yet been fully elucidated, liquid hot water (hydrothermolysis) or flow-through type pretreatment processes have been shown to result in the removal of lignin. This is likely due to the liberation of acids in an autocatalysis type reaction by cleaving the hemiacetal O-acetyl and uronic acid substitutions of the hemicelluloses, resulting in a similar lignin depolymerization reaction pattern as has been observed with steam pretreatment. However, significant lignin removal during liquid hot water pretreatment is usually obtained at the expense of decreased carbohydrate recovery and seems to vary depending on the source of the pretreated biomass (*25*). In the case of alkaline pretreatments such as lime, NaOH or ammonia recycle percolation, lignin is solubilized by the deprotonation of the phenolic lignin subunits combined with alkaline induced breakage of the α and β-aryl ether bonds (*26*). Solvent pretreatments such as ethanol organosolv result in the cleavage of α and β- aryl ether bonds which solubilizes the lignin and increases the free phenolic groups present in the solid substrate (*27*). Although there are not many examples of the effect that the lignin structure in a given pretreated substrate can have on enzymatic hydrolysis, components such as the phenolic groups in lignin are likely to play an important role in lignin cellulase interactions (*28*).

The enzymatic hydrolysis of cellulose is known to be affected by both enzyme- and substrate-related factors (*2a, 29*). Although considerable progress has been made by enzyme companies such as Novozymes and Genencor in reducing the cost of enzymatic hydrolysis (*30*), further improvements are still required. It has been reported that an approximate further 3-fold enzyme cost reduction (from 0.32 to 0.10 $/gal Ethanol) is necessary to reach cost targets for the eventual commercialization of bioconversion of pretreated corn stover to ethanol (*31*).

It is recognized that efficient enzymatic hydrolysis of cellulose is determined by several factors such as the specific surface area, pore size, crystallinity, and degree of polymerization of the cellulose (*2a, 32*). In addition, it is known that the structure and location of hemicellulose and lignin can also affect the hydrolysis efficiency (*2a, 33*). Although hemicellulose has been shown to play a role in limiting accessibility of enzymes to the substrate and in enzyme end product inhibition, of the three main components of lignocellulose, hemicelluloses are known to be the most easy to remove by pretreatment (*34*). Instead, pretreatment studies have tended to focus on minimizing the degradation of hemicelluloses, to products such as furfural and hydroxymethyl furfural, so that the sugars can be obtained in a “useable” form. This can be achieved by using less severe steam pretreatment conditions which, through mild acid hydrolysis (sometimes termed autohydrolysis in unanalyzed conditions) solublizes the hemicellulose resulting in about 80-85% recovery of the original hemicellulose sugars. However, these less severe pretreatment conditions mean that the lignin associated with the cellulose in the water insoluble fraction may still prove to be an impediment to achieving effective enzymatic hydrolysis.

Lignin has been implicated in decreasing the efficiency of enzymatic hydrolysis either by physically decreasing accessibility to or by the adsorption of cellulases (*2a, 10b, 35*). Although the removal of most of the lignin component in lignocellulose is technically possible, using current pulping and/or

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bleaching processes, these processes are currently not economically feasible for bioconversion applications. For example, as the current price of kraft pulp is approximately US $1000 per ton (northern bleached softwood kraft pulp according to the PIX Pulp Benchmark Index), and the theoretical ethanol yields from glucose is 51%, it is clearly not economically feasible to use kraft pulp as a substrate for the ethanol production as the equivalent ethanol would sell for approximately only $350.00 (assuming the bleached pulp has a cellulose content of 90%), without including any costs for making the ethanol from the pulp. The benchmark for producing "cellulosic ethanol" would be to try and make it cost competitive with corn-ethanol which currently sells for approximately $2.26 per gallon.

In summary, it is highly likely that no matter which type of biomass or pretreatment/fractionation process used, the resulting cellulosic substrate will always contain some amounts and types of lignin.

Cellulase-Lignin Interactions

One of the major obstacles to efficient hydrolysis is the fact that cellulases work in a heterogeneous system where the enzymes are soluble while acting on an insoluble substrate. Thus, additional difficulties are presented to the enzymes when they encounter ancillary components of the substrate such as lignin which may restrict their access to cellulose. As mentioned earlier, the two possible mechanisms by which lignin might decrease the yield of cellulose hydrolysis are either "physical", i.e. steric hindrance of the cellulose (*36*) or through the adsorption of cellulases to lignin (either reversibly or irreversibly) rather than cellulose (*10c, 28b, 35a*).

Cellulases have been suggested to adsorb to lignin via hydrophobic (*37*), ionic bond (*10c*) and hydrogen bonding interactions (*10c, 28*). However, the exact mechanisms by which cellulases interact with lignin and result in the reduction in the efficiency of hydrolysis have yet to be fully resolved. Thus, by studying the effects that lignin has on enzymatic hydrolysis this should help us understand both the fundamental, mechanistic enzyme-substrate interactions as well as contributing to the development of efficient pretreatment methods that should improve the action of the cellulase enzymes.

To gain a better sense of how cellulases may potentially interact with lignin we have reviewed some of the general theories that have been advocated to explain protein-solid surface interactions as protein adsorption is known to be affected by the properties of both the protein and the solid surface (*38*). Protein adsorption is a complex process that depends on a number of parameters, such as electrostatic attraction, hydrogen bonding and the hydrophobic/hydrophilic characteristics of both the protein and the surface on which it is adsorbed (*39*). Although protein adsorption seems to primarily involve hydrophobic interactions (*38*, *40*), electrostatic contributions also play an important role, particularly for more hydrophilic surfaces (*38*).

Of the protein properties that can potentially affect adsorption, molecular weight, hydrophobicity, electric charge, and stability are reported to be key (*38*).

Like all proteins, the components of the cellulase enzyme system vary in their characteristics such as their molecular weight, hydrophobicity, electric charge and stability, all of which will affect their adsorption to the lignin component of the pretreated substrates.

Properties of Cellulases

T. reesei has a long history of safe use for industrial enzyme production and is currently used as an important model and commercial system for assessing lignocellulose degradation (*41*). The properties of cellulases from *T. reesei* are summarized in Table I. The molecular weight of the cellulases varies from 23 to 75 kDa and the pI of most cellulases ranges from 3.5 to 7.8. In the case of the *T. reesei* cellulase complex, CBHI and CBHII are the major cellulase components, comprising 60% and 20% respectively of the total protein content (*42*). Most cellulases from *T. reesei* (except EGIII which is < 3% total protein) consist of two domains, a catalytic domain (CD) and cellulose binding domain (CBD), or cellulose biding module (CBM) . The CD works to hydrolyze cellulose into oligosaccharides or glucose, while the CBD or the CBM is thought to increase the adsorption of cellulases onto the surface of cellulose (*43*). The mechanism of cellulase adsorption on pure cellulose has been widely studied and it is reported that the adsorption involves both hydrophobic and hydrogen bonding interactions (*44*). Structure-based site directed mutagenesis studies have indicated that conserved aromatic amino acids are essential for the function of fungal CBD's (*45*). It has also been shown that the CD is involved in adsorption of the enzyme onto lignin (*46*). In addition to the CD, the CBD or the CBM is reported to also play a role in increasing the adsorption of the enzymes onto the lignin (*35a, 47*). The papers cited above describe most of the work that has been carried out on this topic so far and indicates the limited extent of our knowledge with regard to cellulase interactions with solid surfaces. Thus, one of the goals of this paper was to look in more detail at the various charges involved in lignin-cellulose interactions.

Hydrophobic Interactions

Hydrophobic interactions have been shown to be the primary driving force that governs protein adsorption (*38*, *40*). Thus, as the hydrophobicity of both the protein and solid surface increases there is a greater tendency for adsorption to occur as, when protein molecules are dissolved in water, they tend to minimize the exposure of their hydrophobic groups to the aqueous environment (*38*). However, the protein exterior is often partly hydrophobic. Dehydration of hydrophobic portions of the protein and the sorbent surface is driven by entropy gain and, therefore, promotes adsorption to occur rapidly (*38*). As a rule, the amount of protein adsorbed is larger at hydrophobic surfaces.

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Table I. The properties of cellulases from *T. reesei*

Enzyme	*Number of total amino acids*	*Molecular weight (kDa)*	*Isoelectric point (pI)*
CBHI (Cel7A)	497	59-68	3.5-4.2
CBHII (Cel6A)	447	50-58	5.1-6.3
EGI (Cel7B)	437	50-55	3.9-6.0
EGII (Cel5A)	397	48	4.2-5.5
EGIII (Cel12A)	218	25	6.8-7.5
EGV (Cel45A)	225	23	-
β-GI	713	75	7.4-7.8

Actual data was adapted from Hui et. al (2001), Medve et. al (1997), and Valjamae et al. (2001) (*48*).

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As the adsorption of protein onto substrates is generally enhanced by increasing the hydrophobicity of the protein (*49*), it is reasonable to expect that cellulases with different hydrophobicities will adsorb differently onto various lignocellulosic substrates. The hydrophobicity of cellulases can be calculated depending on their amino acid sequence (*50*). The specific amino acids present within some CBM's are reported to promote the adsorption of cellulases on cellulose as well as onto lignin . When Park et al. (2002) modified cellulases with copolymers containing polyoxyalkylene and maleic anhydride (*51*) they found that, as the hydrophilicity of modified cellulases increased, the amounts of free modified enzyme in the supernatant also increased.

In addition to the hydrophobicity of cellulases, the hydrophobicity of the "solid surface" i.e. the lignocellulosic substrate itself, will also affect the tendency of the enzymes adsorb to lignin or cellulose. Consequently, the presence of lignin in the lignocellulose tends to increase the substrates hydrophobicity. When Hodgson and Berg (1988) measured the contact angle of various wood fibers they showed that the contact angle of the α-cellulose was 14.0°, while that of thermomechanical pulps was 42.8° and 51.2°, indicating that the hydrophobicity of lignin is higher than that of cellulose (*52*). When Maximova et al. (*53*) examined the effect of the wetting properties of cellulose fibers and mica (which is a smooth, non-porous, hydrophilic surface), they found that the adsorption of lignin onto cellulose fibers and mica increased the contact angle, indicating that the lignin has a higher hydrophobicity than does the cellulose. When the hydrophobicity of lignin and cellulose were also measured using a thin film prepared by a spin coater (*54*), the contact angle between water and softwood kraft lignin, softwood milled wood lignin (MWL), and hardwood MWL was 46, 52.5, 55.5° respectively, while the contact angle of the cellulose alone was about 20 to 30° (*55*). Thus, pretreated substrates that have a high lignin content will likely be more hydrophobic when compared to cellulose in the absence of lignin. We recently assessed the properties of lignin from steam and organosolv pretreated corn stover, poplar, and lodgepole pine in which we compared lignin yields (*56*). Our work showed that, regardless of the pretreatment method used (steam or organosolv), the isolation of lignin from the pretreated corn stover substrates consistently resulted in lower lignin yields than those of poplar and lodgepole pine. This suggested that the hydrophobicity of the lignin in corn stover substrates was lower when compared to lignins from poplar and lodgepole pine (*56*).

It is apparent that the presence of lignin increases the non-productive adsorption of cellulases and that the hydrophobicity of lignin is higher than that of cellulose, strongly suggesting that hydrophobic interactions are one of the main factors governing the adsorption of cellulases to lignin. When Palonen et al. (2004) prepared three kinds of lignin, (alkali-lignin, cellulolytic enzyme lignin (CEL) lignin and acid-lignin) from steam pretreated spruce (SPS) and compared the adsorption properties of the intact enzymes and the CD from the CBHI and EGII cellulases to the SPS and isolated lignins (*35a*), they showed that the amount of CD adsorbed onto the substrates was lower than observed for the intact enzymes. They thought this was likely due to the greater hydrophobicity of the CBM component of the complete enzyme. As the amount of EGII adsorbed onto the lignins was higher than that of CBHI, they suggested that this difference was a result of the more open nature of the active site of EGII, which lead to greater adsorption through hydrophobic interactions. In related work, the different modules of two cellulase component enzymes, Cel7A (CBHI) and Cel7B (EGI), were compared with regard to their interaction with lignin (*47*). When the full-length enzymes were used, about 50% of the Cel7A and about 65% of the Cel7B were adsorbed onto the isolated lignin from spruce sawdust, while about 15% of the CDs were adsorbed onto the isolated lignin, suggesting that it was primarily the CBMs which contributed to the proteins adsorption onto the lignin. It was also thought that the difference in the hydrophobicity between the CBM of Cel7A and Cel7B was a result of their different adsorption behavior onto the lignin preparations. The CBM of Cel7A has four aromatic residues, all of which are tyrosine. Three of these tyrosines form the flat surface of the CBM giving the enzyme its affinity for crystalline cellulose. The CBM of Cel7B has five aromatic residues, four of which are tyrosine and one is tryptophan. Borjesson et al. (2007) suggested that the presence of a tryptophan in the CBM of Cel7B instead of a tyrosine on the flat surface increased the hydrophobicity of this part of the protein, based on the hydrophobicity scale of amino acid side chains that has been proposed by Roseman (*57*). The difference in the hydrophobicity of the tyrosine is caused by the phenolic hydroxyl group in its side chain, while tryptophan has an indole ring.

In other related work, Berlin et al. (2006) isolated two types of organosolv lignin from Douglas-fir, with one fraction defined as dissolved lignin (DL), as it was precipitated from the organosolv liquor, while the other fractions was termed enzymatic residual lignin (ERL), as it was the insoluble residue remaining after the enzymatic hydrolysis of the organosolv pulp (*10c*). These workers then tried to correlate the functional groups in the lignin preparations with their inhibitory effects on cellulose hydrolysis. The low amounts of carboxyl and aliphatic hydroxyl groups found in the DL when compared to the ERL lignin indicated the higher hydrophobicity of the DL lignin, which inhibited hydrolysis to a greater degree than did the ERL lignin (Table II). However, this observation still needs to be confirmed as the DL was dissolved in 50% (w/w) ethanol while

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the ERL was not dissolved in the ethanol solvent. The empirical parameter, $E_T(30)$ of ethanol/water (80:20), which is similar to the chemical composition of the organosolv washing solvent, is 53.7 kcal/mol, while the $E_T(30)$ of dioxane, which is used for dissolving lignin, is 36.0 kcal/mol (*58*). This means that the hydrophobicity of dioxane is higher than that of the ethanol/water (80:20) mixture. Although these workers did not measure the hydrophobicity of the isolated lignins, it is possible that the hydrophobicity of the DL was lower than that of the ERL.

Other related work has reported that the addition of additives such as Bovine Serum Albumin (BSA), or polyethylene glycol to the reaction mixture can decrease the non-productive binding of cellulases onto lignin (*37a, 47, 59*). Palonen et al. (2004) reported that adding an excess amount of BSA to the cellulose mixture that included CBHI or EGII, resulted in a decrease in the amount of cellulases that bound to the SPS and the CEL-lignin (*35a*). Other workers have shown that cellulases adsorbed to both cellulose and lignin, while BSA adsorbed only onto the lignin (*60*). The reasons for the difference in adsorption properties between the BSA and cellulases are not well understood, although Eriksson et al. (2002) suggested that the BSA has hydrophobic sites, which will bind with fatty acids and will readily adsorb onto hydrophobic surfaces (*37a*).

The addition of surfactants into the reaction mixture has been shown to increase the saccharification of lignocellulosic materials and to decrease the non-productive binding of cellulases to lignin (*37a*). When steam-pretreated spruce substrates were used to compare the action of several non-ionic, anionic, and cationic surfactants for their potential to increase enzymatic hydrolysis (*37a*) the addition of non-ionic or anionic surfactant (sodium dodecyl sulfate; SDS) to the reaction mixture reduced the adsorption amounts of Cel7A onto the SPS. In the related work, Borjesson et al. (2007) showed that non-ionic surfactants and polymers containing poly(ethylene oxide) effectively increased the enzymatic hydrolysis of lignocellulosic substrates (*59*). The adsorption of polyethylene glycol (PEG) onto the SPS was also thought to be through hydrophobic interaction (the dominant action) as well as through hydrogen bond interactions between the PEG and the lignin component in the lignocellulose. It should be noted that the effect of surfactants on hydrolysis was only evident when lignin was present in the substrate (*37a, 59*).

It is most likely that hydrophobic interactions between cellulases and lignin play an important role in the non-productive binding of cellulases, which consequently contributes to the decrease in efficiency of lignocellulosic hydrolysis.

Electrostatic Interactions

Compared to the effect of potential hydrophobic interactions between cellulases and lignin, the role of possible electrostatic effects has not been examined in any detail. In general, both the protein molecule and polymer surface carry charged groups and it is known that dissociation or association of the surface groups is one of the major mechanisms causing a surface charge that originates from the carboxyl, amino, phosphate and imidazole groups. If both acidic and basic groups are present, the surface may become positively or negatively charged, depending on the experimental conditions that are used. Typically, these materials have an electronic neutral point called the pI or point of zero charge. If both the protein molecule and polymer surface have the same charge, they repulse each other. If they possess opposite charges, they adsorb. The maximum amounts of adsorbed protein onto polymers are sometimes the same as the pI of the protein or the pI of protein-polymer complex (*61*). Several groups have looked at possible electrostatic interactions between proteins and solid surfaces. For example, Fukuzaki et al. (1996) examined the interaction between BSA and metal oxide surfaces: silicon dioxide (SiO_2, silica), titanium dioxide (TiO_2, titania), zirconium oxide (ZrO_2, zirconia), and aluminium oxide (Al_2O_3, alumina) (*61a*). The pI of BSA was found to be pH 5.1, at which maximum adsorption was observed for all of the metal oxides. There was a direct correlation between the amount of adsorbed BSA and surface charge density. All of the adsorbed BSA on the metal oxides showed charge shifts towards the positive site, suggesting the possible involvement of the carboxylic acids groups on the BSA molecules. When Elgersma et al. (1990) examined the adsorption of BSA on positively and negatively charged polystyrene lattices with different surface charges (*61b*), they found that maximum adsorption was attained at the pI of the protein-covered polystyrene (PS) particles rather than that of the pI of the protein itself. It is possible that the BSA may be preferentially adsorbed onto lignin when compared to Cel7A, because the pIs of these proteins are quite different (Cel7A: 3.5-4.2, BSA: 4.7-5.3), suggesting that Cel7A carried a negative charge at hydrolysis condition (~pH 5.0), while BSA would carry a positive charge or be electrically neutral.

Table II. Comparison of inhibitory effects on cellulases and amounts of reactive groups in isolated lignins from organsolv pretreated Douglas-fir

	Dissolved Lignin		*Enzymatic residual lignin*
Inhibitory effects on cellulases		>	
CO		>	
COOH		<	
Total OH		<	
Phenolic OH		>	
Aliphatic OH		<	

Actual data was adapted from Berlin et al. (2006) .

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Enzymatic hydrolysis of lignocellulosic substrates is usually carried out under mildly acidic conditions. When the pI values of cellulases are considered (Table I), it is apparent that, under the conditions normally employed for cellulose hydrolysis (pH 4.8-5.0), the CBHII, EGIII, and β-glucosidase components of *T. reesei* will carry a net positive charge and that CBHI, the CBHI core, EGI, and EGII components will carry a net negative charge. The β-glucosidase from *Aspergillus niger*, which is typically added to a reaction mixture to decrease productive inhibition caused by cellobiose, has a pI of 4.0 (*62*). In addition to the net charge of the cellulases, it has been reported that the electric charges of specific amino acids may be involved in the adsorption onto cellulose. When Reinikainen et al. (1995) looked at the interaction between cellulose and the CBHI from *T. reesei* (*44a*), the pH dependency of the adsorbed cellulases indicated that electrostatic repulsion between the bound proteins greatly influenced protein adsorption. As the CBHI core was insensitive to pH, they thought that the observed pH dependent adsorption of the native enzyme was caused by the CBM. When they replaced the amino acid residues in the CBM, a significant reduction in adsorption of the protein to the cellulose was observed. This indicated that, at low pH (<6.2), the histidine (H) 465 which is part of an ionizing amino acid side chain (H465) in the CBM, became positively charged and preferably attracted the hydroxyl oxygen of the tyrosine (Y) 466 side chain. This consequently weakened the hydrogen bond with cellulose, significantly influencing the protein-carbohydrate interaction.

As mentioned earlier, both the cellulases components and the pretreated lignocellulosic substrates possess electrostatic properties which are governed by their aqueous environments. Lignocellulosic fibers carry a negative charge when suspended in water due to the presence and ionization of acidic groups in the hemicelluloses and lignin (*63*). In earlier work, Lin et al. (2008) measured the zeta potential of steam-exploded yellow poplar treated at 240°C for 3 min and subsequently extracted with alkali (*64*). The resulting pulp was shown to have a zeta potential of -14 mV at pH 6.0, with the zeta potential decreasing with increasing pH. They attributed the decrease in the zeta potential to the dissociation of the carboxylic groups and the ionization of the phenolic hydroxyls in the remaining lignin. When Bhardwaj et al. (2004) measured the zeta potential of kraft pulps from *Eucalyptus* (*63*), the zeta potential of unbleached pulps was found to be -32.8 mV and that of the bleached pulp was -13.2 mV. In earlier work, when Dong et al. (1996) measured the zeta potential of Klason lignin from eucalyptus (*65*) the zeta potential of the kraft lignin was shown to be -40 mV at pH 4.5. Thus, it is highly likely that pretreated pulps and lignins carry a negative charge when suspended in water. This assumption is supported by the several studies. When Berlin et al. (2006) measured the chemical groups in lignin from organosolv pulps they found that the isolated lignins contained carboxylic acids (*10c*). Similarly, when Ragnar et al. (2000) measured the pKa-values of guaiacyl- and syringyl-derived phenols the pKa of the phenolic hydroxyl groups were shown to be above 7.0 (*66*), indicating that the phenolic hydroxyl groups are protonated under typical hydrolysis condition (pH 4.8). In related work, when the pKa of carboxylic acid groups were below 5.0, it was shown that the carboxylic acid groups were dissociated in solution (*26*a). In order to determine the role that

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any potential electrostatic interaction between lignin and cellulases might have, the zeta potential of the isolated lignins needs to be measured, to determine if an overall attractive or repulsive force occurs between cellulases and their respective substrates.

As mentioned above, it is highly likely that pretreated substrates will have a negative charge when dispersed in water, primarily because the substrates typically contain significant amounts of carboxyl groups. With the negative charge of the pulps, it is possible that there may be an electrostatic attraction between pretreated substrates and CBHII, EGIII and β-glucosidase from *T. reesei*. On the other hand, there may be an electrostatic repulsion between the substrate and CBHI and EGII from *T. reesei*. The occurrence of electric repulsion between CBHI (Cel7A) from *T. reesei* and an anionic surfactant has been reported previously by Eriksson et al. (2002) (*37a*). They showed that the addition of an anionic surfactant, sodium dodecyl sulfate (SDS), to the reaction mixture reduced the adsorption of Cel7A on the SPS substrate, while the addition of a cationic surfactant increased the Cel7A adsorption onto the SPS. Considering that the pI of the Cel7A is approximately 3.9, the Cel7A must be negative charged under typical hydrolysis conditions (pH 4.8), so that the Cel7A and SDS electrostatically repulse each other. It has been reported that β-glucosidase tend to absorb more onto lignin than do cellulases (*60*). One possible explanation for the adsorption difference is that the β-glucosidase (pI 7.4-7.8) has a net positive charge resulting in a strong attraction to the negatively charged lignin. In addition to the overall electric charge, which is determined by the pI or zeta potential, the partial electric charge caused by specific amino acids has also been reported to influence the amount of cellulase adsorbed onto lignocellulosic substrates (*35a*). Berlin et al. (2006) proposed that ionic-type lignin-enzyme interactions played a major role in protein- substrate interactions as the occurrence of charged (COOH, OH) or partially charged (CO) functional groups on both the lignin and cellulase surfaces could mediate these interactions (*10c*).

Although it is likely that hydrophobic interactions are the major mechanism that determines the amount of adsorbed cellulases, most previous studies (Borjesson et al. 2007; Palonen et al. 2004) did not consider the additional involvement of electrostatic interactions. For example, Palonen et al. (2004) assumed that the different adsorption properties of CBHI and EGII onto lignin were caused by the more “open nature” of the active site of EGII, leading to adsorption through hydrophobic interactions (*35a*). However, it is still possible that the different electric properties between CBHI and EGII caused the observed differences in adsorption behavior. The CBHI would carry a negative charge under the enzymatic hydrolysis conditions because the pI of CBHI ranges from 3.5 to 4.2 (Table I) while EGII would carry a negative (weaker electric charge than CBHI) or positive charge under enzymatic hydrolysis conditions, because the pI of EGII ranges from 4.2 to 5.5 (Table I). It is also possible that the differences in amino acid composition resulted in the different adsorption profiles since the CBM of CBHI has a positively charged amino acid (His) and the CBM of EGII contains no charged amino acids. Borjesson et al. (2007) attributed the different adsorption behavior between CBHI (Cel7A) and EGI (Cel7B) to the difference in hydrophobicity between the enzymes (*47*). However, it is likely that electrostatic interactions are also involved as the pI of EGI (3.9-6.0) indicates that it carries either a lesser negative charge or a positive charge than does the CBHI. In addition, it is apparent that the number of charged amino acids in the respective CBMs are different as there is one positive charged group in the CBM of CBHI, H465 (*44a*), while the number of positive and negative charged amino acids in the CBM of EGI are two and one, respectively (*67*). Recent work by Liu et al. (*68*) showed that the addition of cations to the SPORL pretreated substrates increased the enzymatic hydrolysis of the substrates, suggesting that electrostatic interaction are involved in interaction between lignin and cellulases.

Thus, it is clear that electrostatic interactions are an important factor that influence the amount of protein adsorbed onto solid surfaces (*38*). It is also recognized that the electric charge will vary according to the nature of each of the cellulase components. To determine the electrostatic interactions between cellulases and lignin, the measurement of zeta potential of isolated lignin will be necessary. The reported pI of cellulases varies widely (Table I) and the complex nature of cellulase mixtures will not allow us to easily observe specific cellulase behavior. Therefore, a study on each of the individual cellulases will be needed to be able to provide a greater understanding of enzyme adsorption behavior. If we could determine the adsorption isotherms of cellulases onto isolated lignins as a function of pH, within the pH range normally utilized for hydrolysis reaction, this would greatly enhance our understanding of the role that electrostatic interactions might play between cellulases and lignin.

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Hydrogen Bonding Interactions between Cellulases and Lignin

Another potential mode of interaction between cellulases and lignin is via hydrogen bonding as the hydroxyl groups in cellulose and lignin have been reported to be involved in hydrogen bonding (*28*). Although the carboxylic acid groups in lignin should also be involved in this putative hydrogen bond interaction, only Berlin et al. (2006) have so far mentioned this possibility (*10c*).

There have also been a handful of studies which have mentioned that phenolic hydroxyl groups could be involved in the adsorption of cellulases to lignin. When Sewalt et al (1997) compared the effect of hydroxypropylated organosolv lignin and steam pretreated lignin on filter paper digestion they showed that that the hydroxypropylation of lignin, which removed free phenolic sites, resulted in increased cellulose hydrolysis (*28b*). Pan (2008) used five different isolated lignins: hardwood organosolv lignin, baggasse hydrolytic lignin, softwood kraft lignin, softwood organosolv lignin, and hardwood organosolv lignin to assess the effect of phenolic hydroxyl group on hydrolysis (*28a*). The addition of 20% (w/w) lignin to the reaction mixture containing microcrystalline cellulose (Avicel) resulted in a 10-23% reduction in hydrolysis, depending on the source of the lignin that was used. The hydroxypropylation of the phenolic hydroxyl groups of the isolated lignin reduced the inhibitory effect of lignin, suggesting that the phenolic hydroxyl groups negatively affected the hydrolysis. Pan also used lignin model compounds to assess the role of functional groups within the lignin on enzymatic hydrolysis inhibition. He showed that phenolic compounds resulted in 1-5% more inhibition than did non-phenolic compounds, suggesting that the interference caused by the phenolic hydroxyl groups on lignin during enzymatic hydrolysis played a more important role than did lignin's role in acting as a physical barrier and/or non-specific adsorption. Although hydroxypropylation is a effective method to modify phenolic hydroxyl groups, the hydroxypropylation reaction will likely modify lignin in other ways. For example, the numbers of hydroxyl groups will not be changed as, during the hydroxypropylation reaction, phenolic hydroxyl groups are changed to aliphatic hydroxyl groups. It is also possible that the hydrophobicity increases as a result of the hydroxypropylation reaction due to the addition of the aliphatic carbon. When Berlin et al. (2006) used Nuclear Magnetic Resonance (NMR) to compare the amounts of phenolic hydroxyl groups in the DL and ERL lignin from organosolv pretreated softwood substrates they found that the DL decreased the hydrolysis more than did the ERL while the phenolic hydroxyl content was higher in the DL than in the ERL (*10c*). This indicated that the phenolic hydroxyl groups negatively affected the hydrolysis. One study that did not find a correlation between the amount of phenolic hydroxyl groups in lignin and adsorption of protein was the work of Kawamoto et al. (1992) who examined the adsorption of BSA on five lignin preparation: thiolignin, steam-explosion lignin, acetic acid lignin, organosolv lignin, and MWL (*69*). Although the phenolic hydroxyl groups were present at almost the same level (about 0.3/C_6-C_3 unit) for each of the five lignin preparations, the amount of adsorbed of BSA was different. They therefore concluded that more complex factors, other than just the content of the phenolic hydroxyl groups, influenced the protein-adsorbing ability of lignin.

In addition to phenolic hydroxyl groups, aliphatic hydroxyl groups have also been shown to be involved in hydrogen bonding (*70*). The CBM of cellulases are known to interact with the hydroxyl groups on cellulose, indicating that aliphatic hydroxyl groups are also involved in the adsorption on lignin. However, the role of aliphatic hydroxyl groups in the binding between cellulases and lignin has yet to be reported. When Borjesson et al. (2007) used surfactant MEGA 10 containing aliphatic hydroxyl groups (*47*), they were able to increase the hydrolysis of steam pretreatment spruce from 47% to 65%. When Berlin et al. (2006) compared aliphatic hydroxyl groups in the isolated two lignins from Douglas-fir (*10c*), the aliphatic hydroxyl groups content was higher in the ERL than in the DL, whereas the inhibitory effects on the cellulase preparations was greater with DL than with the ERL. However, this was likely due to the DL having a lower hydrophobicity when compared to the ERL.

It is known that hydroxyl groups are involved in hydrogen bonding (*70*) and phenolic hydroxyl groups and aliphatic hydroxyl groups in lignin could, hypothetically, be involved in the hydrogen bonding. As mentioned earlier, Sewalt et al. (2008) and Pan (2008) reported that hydroxypropylation reduced the negative effect of lignin on the hydrolysis reaction (*28*), while Kawamoto et al. (1992) reported that there was no correlation between the amount of phenolic hydroxyl groups and the adsorbed protein content (*69*). The presence of carboxylic groups also should be considered as these groups will also increase the hydrogen bond interaction (*70*).

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Steric or Physical Blocking of Cellulose by Lignin

In addition to non-productively binding cellulases, lignin also contributes to physically blocking cellulose thereby preventing cellulases from accessing cellulose. There have been limited studies on the physical effects of lignin vs non-productive binding of cellulases to lignin since for a given substrate, it is difficult to delineate between these contributions of lignin toward reducing enzymatic hydrolysis yields. It should also be noted that the lignin content itself is not necessarily a key indicator of the negative effect of lignin on hydrolysis as the location of lignin is likely a key determinant in the role it plays in limiting enzymatic hydrolysis.

For example, it has been shown that increasing steam pretreatment severity in *Pinus radiata* and lodgepole pine (*Pinus contorta*) results in an increased substrate lignin content but also increases the celluose enzymatic digestibility. This has been attributed to a redistribution of lignin as the severity is increased, resulting in increasing substrate accessibility (*71*) . Some earlier attempts to remove lignin from pretreated substrates via 'post treatments" such as NaOH extraction have sometimes resulted in "negative" lignin redistribution which decreased enzymatic hydrolysis even though the total amount of lignin in the substrate decreased (*72*). It has been suggested that pretreatments such as steam (*72*), hydrothermal (*73*) and dilute acid (*24*) result in lignin flow, thus redistributing the lignin from the fiber wall to fiber surface or to form globular formations at the fiber surface which are redeposited onto the surface thus posing both physical and chemical obstructions to the cellulases (*74*). Microscopy studies which have visualized the lignin droplets on the surface of pretreated substrates have shown that these droplets have a negative effect on the enzymatic hydrolysis, which increases concomitantly with an increase in the number of droplets detected per unit area of substrate surface (*24*).

Lignin has also been shown to restrict the swelling of lignocellulosic substrates and reducing pore volume (*75*). Due to its hydrophobic nature it is likely that an increase in the lignin covering the carbohydrate components of a given substrate would result in a decrease in overall hydrophilicity and "swelling" of the substrate in water. For example, sulfonation of a highly lignified RMP pulp sample showed an increase in hydrolysis yields from approximately 20 to 50%, by improving swelling and likely increasing the charge properties of the substrate lignin (*36*). However, the sulfonate pulp was not completely hydrolyzed despite these improvements, indicating that the lignin still played a role in physically blocking the cellulose, as a subsequent delignification treatment of the RMP resulted in complete hydrolysis of the cellulose (*36*). Further elucidating the relative magnitude of the contributions of the physical and chemical interactions of lignin with cellulases is a key area in need of further investigation, especially for pretreatments which do not result in significant removal of the lignin from the biomass substrate.

Conclusions

Biomass-to-ethanol processes require effective pretreatment and enzymatic hydrolysis of the cellulose. Lignin, one of the main components of lignocellulosic substrates, decreases the efficiency of enzymatic hydrolysis of pretreated substrates by steric hindrance (preventing swelling of the substrates and by "getting in the way") and by non-productive binding of cellulases. Although hydrophobic, electrostatic, and hydrogen bonding interaction have been suggested to be involved in the interaction between cellulases and lignin, specific mechanisms for these interactions have yet to be fully elucidated because of the variability of biomass substrates, pretreatments and conditions employed. It is apparent that each cellulase component varies in its hydrophobicity and electrostatic properties. However, hydrophobic and electrostatic interactions seem to play a significant role in determining the amount of non-productive binding of cellulases to lignin during enzymatic hydrolysis when compared to the influence of hydrogen bonding interactions. It is likely that a cost effective pretreatment and fractionation process that removes all lignin from the biomass substrate will prove elusive to find! Thus we need to still better understand the role that lignin plays in limiting accessibility of the enzymes to the cellulose through its influence on both steric/swelling and protein adsorption roles.

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References

1. (a) Himmel, M. E.; Ding, S. Y.; Johnson, D. K.; Adney, W. S.; Nimlos, M. R.; Brady, J. W.; Foust, T. D. Biomass recalcitrance: Engineering plants and enzymes for biofuels production. *Science* **2007**, *315* (5813), 804−807. (b) Mabee, W. E.; Saddler, J. N. Bioethanol from lignocellulosics: Status and perspectives in Canada. *Bioresour. Technol.* **2010**, *101* (13), 4806−4813. (c) Farrell, A. E.; Plevin, R. J.; Turner, B. T.; Jones, A. D.; O'Hare, M.; Kammen, D. M. Ethanol can contribute to energy and environmental goals. *Science* **2006**, *311* (5760), 506−508.
2. (a) Chandra, R. P.; Bura, R.; Mabee, W. E.; Berlin, A.; Pan, X.; Saddler, J. N. Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics? *Adv. Biochem. Eng. Biotechnol.* **2007**, *108*, 67−93. (b) Hoekman, S. K. Biofuels in the U.S.: Challenges and opportunities. *Renewable Energy* **2009**, *34* (1), 14−22. (c) Sims, R. E. H.; Mabee, W.; Saddler, J. N.; Taylor, M. An overview of second generation biofuel technologies. *Bioresour. Technol.* **2010**, *101* (6), 1570−1580.
3. (a) IEA Biofuel production. www.iea.org/Textbase/techno/essentials.htm (accessed 13 Jan. 2011). (b) Banerjee, S.; Mudliar, S.; Sen, R.; Giri, B.; Satpute, D.; Chakrabarti, T.; Pandey, R. Commercializing lignocellulosic bioethanol: Technology bottlenecks and possible remedies. *Biofuels, Bioprod. Biorefin.* **2010**, *4* (1), 77−93.
4. (a) Sjostorm, E. *Wood Chemistry: Fundamentals and Applications*, 2nd edition; Academic Press: Washington DC, 1981. (b) Jørgensen, H.; Kristensen, J. B.; Felby, C. Enzymatic conversion of lignocellulose into

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch006

fermentable sugars: challenges and opportunities. *Biofuels, Bioprod. Biorefin.* **2007**, *1* (2), 119−134.

5. (a) Wyman, C. *Handbook on Bioethanol: Production and Utilization*; Taylor & Francis: 1996. (b) Galbe, M.; Zacchi, G. A review of the production of ethanol from softwood. *Appl. Microbiol. Biotechnol.* **2002**, *59* (6), 618−628.
6. Baucher, M.; Monties, B.; Van Montagu, M.; Boerjan, W. Biosynthesis and genetic engineering of lignin. *Crit. Rev. Plant Sci.* **1998**, *17* (2), 125–197.
7. Saulnier, L.; Crepeau, M. J.; Lahaye, M.; Thibault, J. F.; Garcia-Conesa, M. T.; Kroon, P. A.; Williamson, G. Isolation and structural determination of two 5,5′-diferuloyl oligosaccharides indicate that maize heteroxylans are covalently cross-linked by oxidatively coupled ferulates. *Carbohydr. Res.* **1999**, *320* (1-2), 82–92.
8. Ohgren, K.; Bura, R.; Saddler, J.; Zacchi, G. Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. *Bioresour. Technol.* **2007**, *98* (13), 2503–2510.
9. Selig, M. J.; Knoshaug, E. P.; Adney, W. S.; Himmel, M. E.; Decker, S. R. Synergistic enhancement of cellobiohydrolase performance on pretreated corn stover by addition of xylanase and esterase activities. *Bioresour. Technol.* **2008**, *99* (11), 4997–5005.
10. (a) Bura, R.; Chandra, R.; Saddler, J. Influence of Xylan on the enzymatic hydrolysis of steam-pretreated corn stover and hybrid poplar. *Biotechnol. Prog.* **2009**, *25* (2), 315−322. (b) Kumar, R.; Wyman, C. E. Access of cellulase to cellulose and lignin for poplar solids produced by leading pretreatment technologies. *Biotechnol. Prog.* **2009**, *25* (3), 807−819. (c) Berlin, A.; Balakshin, M.; Gilkes, N.; Kadla, J.; Maximenko, V.; Kubo, S.; Saddler, J. Inhibition of cellulase, xylanase and beta-glucosidase activities by softwood lignin preparations. *J. Biotechnol.* **2006**, *125* (2), 198−209.
11. (a) Kim, T. H.; Lee, Y. Y. Pretreatment and fractionation of corn stover by ammonia recycle percolation process. *Bioresour. Technol.* **2005**, *96* (18), 2007−2013. (b) Kumar, L.; Chandra, R.; Chung, P. A.; Saddler, J. Can the same steam pretreatment conditions be used for most softwoods to achieve good, enzymatic hydrolysis and sugar yields? *Bioresour. Technol.* **2010**, *101* (20), 7827−7833.
12. Kumar, P.; Barrett, D. M.; Delwiche, M. J.; Stroeve, P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind. Eng. Chem. Res.* **2009**, *48* (8), 3713–3729.
13. Shevchenko, S. M.; Chang, K.; Dick, D. G.; Gregg, D. J.; Saddler, J. N. Structure and properties of lignin in softwoods after SO_2-catalyzed steam explosion and enzymatic hydrolysis. *Cell Chem. Technol.* **2001**, *35* (5−6), 487–502.
14. Boussaid, A. L.; Esteghlalian, A. R.; Gregg, D. J.; Lee, K. H.; Saddler, J. N. Steam pretreatment of Douglas-fir wood chips: Can conditions for optimum hemicellulose recovery still provide adequate access for efficient enzymatic hydrolysis? *Appl. Biochem. Biotechnol.* **2000**, *84−86*, 693–705.
15. (a) Neilson, M. J.; Shafizadeh, F.; Aziz, S.; Sarkanen, K. V. Evaluation of organosolv pulp as a suitable substrate for rapid enzymatic-hydrolysis. *Biotechnol. Bioeng.* **1983**, *25* (2), 609−612. (b) Pan, X. J.; Arato, C.;

Gilkes, N.; Gregg, D.; Mabee, W.; Pye, K.; Xiao, Z. Z.; Zhang, X.; Saddler, J. Biorefining of softwoods using ethanol organosolv pulping: Preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products. *Biotechnol. Bioeng.* **2005**, *90* (4), 473−481.

16. Wyman, C. E.; Dale, B. E.; Elander, R. T.; Holtzapple, M.; Ladisch, M. R.; Lee, Y. Y. Coordinated development of leading biomass pretreatment technologies. *Bioresour. Technol.* **2005**, *96* (18), 1959–1966.
17. (a) Kaar, W. E.; Holtzapple, M. T. Using lime pretreatment to facilitate the enzymic hydrolysis of corn stover. *Biomass Bioenerg.* **2000**, *18* (3), 189−199. (b) Chang, V. S.; Nagwani, M.; Kim, C. H.; Holtzapple, M. T. Oxidative lime pretreatment of high-lignin biomass: Poplar wood and newspaper. *Appl. Biochem. Biotechnol.* **2001**, *94* (1), 1−28.
18. Saha, B. C.; Iten, L. B.; Cotta, M. A.; Wu, Y. V. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. *Process Biochem.* **2005**, *40* (12), 3693–3700.
19. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y. Y.; Holtzapple, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* **2005**, *96* (6), 673–686.
20. Sanchez, O. J.; Cardona, C. A. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour. Technol.* **2008**, *99* (13), 5270–5295.
21. Wyman, C. E.; Dale, B. E.; Elander, R. T.; Holtzapple, M.; Ladisch, M. R.; Lee, Y. Y. Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn stover. *Bioresour. Technol.* **2005**, *96* (18), 2026–2032.
22. (a) Sarkanen, S.; Teller, D. C.; Hall, J.; McCarthy, J. L. Lignin. 18. Associative effects among organosolv lignin components. *Macromolecules* **1981**, *14* (2), 426−434. (b) Pan, X.; Gilkes, N.; Kadla, J.; Pye, K.; Saka, S.; Gregg, D.; Ehara, K.; Xie, D.; Lam, D.; Saddler, J. Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: Optimization of process yields. *Biotechnol. Bioeng.* **2006**, *94* (5), 851−61.
23. (a) Sannigrahi, P.; Ragauskas, A. J.; Miller, S. J. Effects of two-stage dilute acid pretreatment on the structure and composition of lignin and cellulose in loblolly pine. *Bioenergy Res.* **2008**, *1* (3−4), 205−214. (b) Li, J.; Henriksson, G.; Gellerstedt, G. Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. *Bioresour. Technol.* **2007**, *98* (16), 3061−3068. (c) Shevchenko, S. M.; Beatson, R. P.; Saddler, J. N. The nature of lignin from steam explosion enzymatic hydrolysis of softwood: Structural features and possible uses. *Appl. Biochem. Biotechnol.* **1999**, *77−79*, 867−876.
24. Selig, M. J.; Viamajala, S.; Decker, S. R.; Tucker, M. P.; Himmel, M. E.; Vinzant, T. B. Deposition of lignin droplets produced during dilute acid pretreatment of maize stems retards enzymatic hydrolysis of cellulose. *Biotechnol. Prog.* **2007**, *23* (6), 1333–1339.
25. (a) Mok, W. S. L.; Antal, M. J. Uncatalyzed solvolysis of whole biomass hemicellulose by hot compressed liquid water. *Ind. Eng. Chem. Res.* **1992**,

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch006

31 (4), 1157−1161. (b) Bobleter, O.; Concin, R. Degradation of poplar lignin by hydrothermal treatment. *Cell Chem. Technol.* **1979**, *13*, 583−593.

26. (a) Sjostorm, E. The origin of charge on cellulosic fibers. *Nord. Pulp. Pap. Res. J.* **1989**, *4* (2), 90−93. (b) Yan, J. F. Kinetics of delignication: A molecular approach. *Science* **1982**, *215* (4538), 1390−1392. (c) Gierer, J. Reactions of lignin during pulping: A description and comparison of conventional pulping processes. *Sven. Papperstidn.* **1970**, *73* (18), 571−599.
27. (a) Aziz, S.; Sarkanen, K. Organosolv pulping: A review. *Tappi J.* **1989**, *72* (3), 169−175. (b) Anderson, W. F.; Akin, D. E. Structural and chemical properties of grass lignocelluloses related to conversion for biofuels. *J. Ind. Microbiol. Biot.* **2008**, *35* (5), 355−366.
28. (a) Pan, X. J. Role of functional groups in lignin inhibition of enzymatic hydrolysis of cellulose to glucose. *J. Biobased Mater. Bioenergy* **2008**, *2* (1), 25−32. (b) Sewalt, V. J. H.; Glasser, W. G.; Beauchemin, K. A. Lignin impact on fiber degradation. 3. Reversal of inhibition of enzymatic hydrolysis by chemical modification of lignin and by additives. *J. Agric. Food Chem.* **1997**, *45* (5), 1823−1828.
29. (a) Mansfield, S. D.; Mooney, C.; Saddler, J. N. Substrate and enzyme characteristics that limit cellulose hydrolysis. *Biotechnol. Prog.* **1999**, *15* (5), 804−816. (b) Zhang, Y. H. P.; Lynd, L. R. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems. *Biotechnol. Bioeng.* **2004**, *88* (7), 797−824.
30. (a) Novozymes, Novozymes and NREL Reduce Enzyme Cost. Press release, 2005. (b) Aden, A.; Foust, T. Technoeconomic analysis of the dilute sulfuric acid and enzymatic hydrolysis process for the conversion of corn stover to ethanol. *Cellulose* **2009**, *16* (4), 535−545. (c) Merino, S. T.; Cherry, J. Progress and challenges in enzyme development for Biomass utilization. *Adv. Biochem. Eng. Biot.* **2007**, *108*, 95−120.
31. Aden, A. Biochemical Production of Ethanol from Corn Stover: 2007 State of Technology Model; Technical Report NREL/TP-510-43205; 2008.
32. (a) Grethlein, H. E. The effect of pore-size distribution on the rate of enzymatic-hydrolysis of cellulosic substrates. *Bio-Technology* **1985**, *3* (2), 155−160. (b) Grous, W. R.; Converse, A. O.; Grethlein, H. E. Effect of steam explosion pretreatment on pore-size and enzymatic-hydrolysis of Poplar. *Enzyme Microb. Technol.* **1986**, *8* (5), 274−280. (c) Shevchenko, S. M.; Chang, K.; Robinson, J.; Saddler, J. N. Optimization of monosaccharide recovery by post-hydrolysis of the water-soluble hemicellulose component after steam explosion of softwood chips. *Bioresour. Technol.* **2000**, *72* (3), 207−211. (d) Mooney, C. A.; Mansfield, S. D.; Beatson, R. P.; Saddler, J. N. The effect of fiber characteristics on hydrolysis and cellulase accessibility to softwood substrates. *Enzyme Microb. Technol.* **1999**, *25* (8−9), 644−650.
33. Zhu, J. Y.; Zhu, W. Y.; Obryan, P.; Dien, B. S.; Tian, S.; Gleisner, R.; Pan, X. J. Ethanol production from SPORL-pretreated lodgepole pine: preliminary evaluation of mass balance and process energy efficiency. *Appl. Microbiol. Biotechnol.* **2010**, *86* (5), 1355–1365.

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34. Bura, R.; Mansfield, S. D.; Saddler, J. N.; Bothast, R. J. SO_2-catalyzed steam explosion of corn fiber for ethanol production. *Appl. Biochem. Biotechnol.* **2002**, *98*, 59–72.
35. (a) Palonen, H.; Tjerneld, F.; Zacchi, G.; Tenkanen, M. Adsorption of *Trichoderma reesei* CBH I and EG II and their catalytic domains on steam pretreated softwood and isolated lignin. *J. Biotechnol.* **2004**, *107* (1), 65−72. (b) Grabber, J. H. How do lignin composition, structure, and cross-linking affect degradability? A review of cell wall model studies. *Crop Sci.* **2005**, *45* (3), 820.
36. Mooney, C. A.; Mansfield, S. D.; Touhy, M. G.; Saddler, J. N. The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwoods. *Bioresour. Technol.* **1998**, *64* (2), 113–119.
37. (a) Eriksson, T.; Borjesson, J.; Tjerneld, F. Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. *Enzyme Microb. Technol.* **2002**, *31* (3), 353−364. (b) Ooshima, H.; Sakata, M.; Harano, Y. Enhancement of enzymatic-hydrolysis of cellulose by surfactant. *Biotechnol. Bioeng.* **1986**, *28* (11), 1727−1734.
38. Norde, W. Adsorption of proteins from solution at the solid-liquid interface. *Adv. Colloid Interface Sci.* **1986**, *25* (4), 267–340.
39. Haynes, C. A.; Norde, W. Globular proteins at solid/liquid interfaces. *Colloids Surf., B* **1994**, *2*, 517–566.
40. Schmaier, A. H.; Silver, L.; Adams, A. L.; Fischer, G. C.; Munoz, P. C.; Vroman, L.; Colman, R. W. The effect of high molecular weight kinnigen on surface adsorbed fibrinogen. *Thromb. Res.* **1984**, *33* (1), 51–67.
41. Zhang, Y. H. P.; Himmel, M. E.; Mielenz, J. R. Outlook for cellulase improvement: Screening and selection strategies. *Biotechnol. Adv.* **2006**, *24* (5), 452–481.
42. Goyal, A.; Ghosh, B.; Eveleigh, D. Characteristics of fungal cellulases. *Bioresour. Technol.* **1991**, *36* (1), 37–50.
43. Tu, M. B.; Chandra, R. P.; Saddler, J. N. Recycling cellulases during the hydrolysis of steam exploded and ethanol pretreated lodgepole pine. *Biotechnol. Prog.* **2007**, *23* (5), 1130–1137.
44. (a) Reinikainen, T.; Teleman, O.; Teeri, T. T. Effects of pH and high inoic-strength on the adsorption and activity of native and mutated cellobiohydrolase I from *Trichoderma reesei*. *Proteins: Struct., Funct., Genet.* **1995**, *22* (4), 392−403. (b) Baker, C. S.; Suvajittanont, W.; Bothwell, M. K.; McGuire, J. Adsorption of *Thermomonospora fusca* E-5 and *Trichoderma reesei* cellobiohydrolase I cellulases on synthetic surfaces. *Appl. Biochem. Biotechnol.* **2001**, *94* (1), 29−40.
45. Reinikainen, T.; Ruohonen, L.; Nevanen, T.; Laaksonen, L.; Kraulis, P.; Jones, T. A.; Knowles, J. K. C.; Teeri, T. T. Investigation of the function of mutated cellulose-binding domains of *Trichoderma reesei* Cellobiohydrolase I. *Proteins: Struct., Funct., Genet.* **1992**, *14* (4), 475–482.
46. Berlin, A.; Gilkes, N.; Kurabi, A.; Bura, R.; Tu, M.; Kilburn, D.; Saddler, J. Weak lignin-binding enzymes: A novel approach to improve activity of cellulases for hydrolysis of lignocellulosics. *Appl. Biochem. Biotechnol.* **2005**, *121-124*, 163–170.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch006

47. Borjesson, J.; Engqvist, M.; Sipos, B.; Tjerneld, F. Effect of poly(ethylene glycol) on enzymatic hydrolysis and adsorption of cellulase enzymes to pretreated lignocellulose. *Enzyme Microb. Technol.* **2007**, *41* (1-2), 186–195.
48. (a) Hui, J. P. M.; Lanthier, P.; White, T. C.; McHugh, S. G.; Yaguchi, M.; Roy, R.; Thibault, P. Characterization of cellobiohydrolase I (Cel7A) glycoforms from extracts of *Trichoderma reesei* using capillary isoelectric focusing and electrospray mass spectrometry. *J. Chromatogr., B* **2001**, *752* (2), 349−368. (b) Medve, J.; Stahlberg, J.; Tjerneld, F., Isotherms for adsorption of cellobiohydrolase I and II from *Trichoderma reesei* on microcrystalline cellulose. *Appl. Biochem. Biotechnol.* **1997**, *66* (1), 39−56. (c) Valjamae, P.; Pettersson, G.; Johansson, G., Mechanism of substrate inhibition in cellulose synergistic degradation. *Eur. J. Biochem.* **2001**, *268* (16), 4520−4526.
49. Hansen, J.; Ely, K.; Horsley, D.; Herron, J.; Hlady, V.; Andrade, J. D. The adsorption of lysozymes: A model system. *Makromol. Chem., Macromol. Symp.* **1988**, *17*, 135–154.
50. Yemshanov, D.; McKenney, D. Fast-growing poplar plantations as a bioenergy supply source for Canada. *Biomass Bioenerg.* **2008**, *32* (3), 185–197.
51. Park, J. W.; Park, K.; Song, H.; Shin, H. Saccharification and adsorption characteristics of modified cellulases with hydrophilic/hydrophobic copolymers. *J. Biotechnol.* **2002**, *93* (3), 203–208.
52. Hodgson, K. T.; Berg, J. C. Dynamic wettability properties of single wood pulp fibers and their relationship to absorbency. *Wood Fiber Sci.* **1988**, *20* (1), 3–17.
53. Maximova, N.; Osterberg, M.; Laine, J.; Stenius, P. The wetting properties and morphology of lignin adsorbed on cellulose fibres and mica. *Colloids Surf., A* **2004**, *239* (1-3), 65–75.
54. (a) Notley, S. M.; Norgren, M. Surface energy and wettability of spin-coated thin films of lignin isolated from wood. *Langmuir* **2010**, *26* (8), 5484−5490. (b) Norgren, M.; Gardlund, L.; Notley, S. M.; Htun, M.; Wagberg, L. Smooth model surfaces from lignin derivatives. II. Adsorption of polyelectrolytes and PECs monitored by QCM-D. *Langmuir* **2007**, *23* (7), 3737−3743. (c) Norgren, M.; Notley, S. M.; Majtnerova, A.; Gellerstedt, G. Smooth model surfaces from lignin derivatives. I. Preparation and characterization. *Langmuir* **2006**, *22* (3), 1209−1214.
55. (a) Eriksson, M.; Notley, S. M.; Wågberg, L. Cellulose thin films: Degree of cellulose ordering and its influence on adhesion. *Biomacromolecules* **2007**, *8* (3), 912−919. (b) Gunnars, S.; Wagberg, L.; Stuart, M. A. C. Model films of cellulose: I. Method development and initial results. *Cellulose* **2002**, *9* (3−4), 239−249.
56. Nakagame, S.; Chandra, R. P.; Saddler, J. N. The effect of isolated lignins, obtained from a range of pretreated lignocellulosic substrates, on enzymatic hydrolysis. *Biotechnol. Bioeng.* **2010**, *105* (5), 871–879.
57. Roseman, M. A. Hydrophilicity of polar amino acid side chains is markedly reduced by flanking peptide bonds. *J. Mol. Biol.* **1988**, *200* (3), 513–522.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch006

58. Reichardt, C. Empirical parameters of solvent polarity as liner free-energy relationships. *Angew. Chem., Int. Ed. Engl.* **1979**, *18* (2), 98–110.
59. Borjesson, J.; Peterson, R.; Tjerneld, F. Enhanced enzymatic conversion of softwood lignocellulose by poly(ethylene glycol) addition. *Enzyme Microb. Technol.* **2007**, *40* (4), 754–762.
60. Yang, B.; Wyman, C. E. BSA treatment to enhance enzymatic hydrolysis of cellulose in lignin containing substrates. *Biotechnol. Bioeng.* **2006**, *94* (4), 611–617.
61. (a) Fukuzaki, S.; Urano, H.; Nagata, K. Adsorption of bovine serum albumin onto metal oxide surfaces. *J. Ferment. Bioeng.* **1996**, *81* (2), 163−167. (b) Elgersma, A. V.; Zsom, R. L. J.; Norde, W.; Lyklema, J. The adsorption of bovine serum-albumin on positively and negatively charged polystyrene lattices. *J. Colloid Interface Sci.* **1990**, *138* (1), 145−156.
62. McCleary, B. V.; Harrington, J. Purification of beta-D-glucosidase from *Aspergillus niger*. *Methods Enzymol.* **1988**, *160*, 575–583.
63. Bhardwaj, N. K.; Kumar, S.; Bajpai, P. K. Effects of processing on zeta potential and cationic demand of kraft pulps. *Colloids Surf., A* **2004**, *246* (1−3), 121–125.
64. Lin, Z. Y.; Renneckar, S.; Hindman, D. P. Nanocomposite-based lignocellulosic fibers 1. Thermal stability of modified fibers with clay-polyelectrolyte multilayers. *Cellulose* **2008**, *15* (2), 333–346.
65. Dong, D. J.; Fricke, A. L.; Moudgil, B. M.; Johnson, H. Electrokinetic study of kraft lignin. *Tappi J.* **1996**, *79* (7), 191–197.
66. Ragnar, M.; Lindgren, C. T.; Nilvebrant, N. O. pKa-values of guaiacyl and syringyl phenols related to lignin. *J. Wood Chem. Technol.* **2000**, *20* (3), 277–305.
67. Gilkes, N. R.; Henrissat, B.; Kilburn, D. G.; Miller, R. C.; Warren, R. A. J. Domains in microbial beta-1,4-glycanases -sequence conservation, function, and enzyme families. *Microbiol. Rev.* **1991**, *55* (2), 303–315.
68. (a) Liu, H.; Zhu, J. Y. Eliminating inhibition of enzymatic hydrolysis by lignosulfonate in unwashed sulfite-pretreated aspen using metal salts. *Bioresour. Technol.* **2010**, *101* (23), 9120−9127. (b) Liu, H.; Zhu, J. Y.; Fu, S. Y. Effects of lignin-metal complexation on enzymatic hydrolysis of cellulose. *J. Agric. Food Chem.* **2010**, *58* (12), 7233−7238.
69. Kawamoto, H.; Nakatsubo, F.; Murakami, K. Protein-adsorbing capacities of lignin samples. *Mokuzai Gakkaishi* **1992**, *38* (1), 81–84.
70. Brey, W. S. *Physical Chemistry and Its Biological Applications*; Academic Press, Inc.: New York, 1978.
71. (a) Wong, K. K. Y.; Deverell, K. F.; Mackie, K. L.; Clark, T. A.; Donaldson, L. A. The relationship between fiber porosity and cellulose digestibility in steam-exploded *Pinus radiata*. *Biotechnol. Bioeng.* **1988**, *31* (5), 447−456. (b) Chandra, R. P.; Ewanick, S. M.; Chung, P. A.; Au-Yeung, K.; Del Rio, L.; Mabee, W.; Saddler, J. N. Comparison of methods to assess the enzyme accessibility and hydrolysis of pretreated lignocellulosic substrates. *Biotechnol. Lett.* **2009**, *31* (8), 1217−1222.
72. Donaldson, L. A.; Wong, K. K. Y.; Mackie, K. L. Ultrastructure of steam-exploded wood. *Wood Sci. Technol.* **1988**, *22* (2), 103–114.

73. Kristensen, J. B.; Thygesen, L. G.; Felby, C.; Jorgensen, H.; Elder, T. Cell-wall structural changes in wheat straw pretreated for bioethanol production. *Biotechnol. Biofuels* **2008**, *1*.
74. (a) Vignon, M. R.; GarciaJaldon, C.; Dupeyre, D. Steam explosion of woody hemp chenevotte. *Int. J. Biol. Macromol.* **1995**, *17* (6), 395−404. (b) Michalowicz, G.; Toussaint, B.; Vignon, M. R. Ultrastructural changes in poplar cell wall during steam explosion treatment *Holzforschung* **1991**, *45* (3), 175−179.
75. (a) Stone, J. E.; Scallan, A. M., Effect of component removal upon porous structure of cell wall of wood. *J. Polym. Sci., Part C: Polym. Symp.* **1965**, *11* (1), 13−25. (b) Eriksson, I.; Haglind, I.; Lidbrandt, O.; Sahnén, L. Fiber swelling favoured by lignin softening. *Wood Sci. Technol.* **1991**, *25* (2), 135−144.

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Chapter 7

Approaches To Deal with Toxic Inhibitors during Fermentation of Lignocellulosic Substrates

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The recalcitrance of lignocellulose materials requires harsh pretreatment(s) to release sugar monomers for ethanol fermentation by microorganisms. Harsh pretreatment conditions result in the formation of compounds in the hydrolysate that are inhibitory to the fermenting microorganism(s). The main inhibitors include furan derivatives, organic acids, and phenolic compounds. Research has focused on physical, chemical and/or biological methods to deal with inhibitors. Many of these methods remove or convert inhibitors through a detoxification step prior to fermentation. Recently, biological methods have focused on increasing yeast inhibitor tolerance. This review examines research on the different methods used to detoxify hydrolysates or increase yeast tolerance to inhibitors.

Introduction

Concerns over the effect of greenhouse gas (GHG) emissions and rising oil prices have led to efforts to develop clean and/or renewable fuel sources. Many industrialized countries, including Canada, have committed to the Kyoto Protocol which requires that, by 2012, emissions of carbon dioxide and other GHG be reduced to 6% below 1990 levels. In Canada, the transportation sector contributes 27% of the total GHG emissions (*1*). The Canadian government has mandated 5% renewable fuel content in gasoline and 2% content in diesel and home heating fuels by 2011. In total, 2.6 billion litres of renewable fuel will need to be produced with the goal of reducing GHG emissions by 4 million tons/year (*2*).

Ethanol is an appealing alternative energy source that burns more completely than gasoline (*3*), making it both a substitute and additive for petroleum-derived fuels. Adding ethanol to gasoline oxygenates the fuel, allowing more complete combustion and reduced emission of pollutants. In Canada, gasoline mixtures containing up to 10% ethanol are typically used.

In Canada, the storage carbohydrates in agricultural crops such as corn and wheat are used as substrates for bioethanol production (*4*). These crops are traditionally grown for food and animal feed, and may not be sustainable for large-scale ethanol production (*5*, *6*). In contrast, vast quantities of sugars occur as structural polysaccharides in the form of cellulose and hemicellulose in lignocellulosic biomass residues. Lignocellulosic residues are more abundant than food crops, have fewer competing uses, and can be harvested with considerably less interference to the food economy and less impact on environmental resources. Using today's technology, the bioconversion of lignocellulosic sugars to ethanol is comparatively difficult and expensive relative to starch conversion. Thus, there is interest to refine existing technology or develop new technologies to produce the second generation "lignocellulosic" ethanol to provide the large volume needed to satisfy the demand for the transportation sector.

Lignocellulosic substrates envisioned for bioconversion include crop residues, municipal solid wastes, forestry residues and discarded material from paper mills. These low-value waste materials can be converted to produce ethanol. Lignocellulosic biomass is comprised of lignin (10-25%) as well as the structural carbohydrates hemicellulose (20-35%) and cellulose (35-50%) (*7*, *8*). The composition of various lignocellulosic materials is summarized in Table I. Cellulose is a linear polymer of glucose molecules linked by β-1, 4 glycosidic bonds, making it highly crystalline, compact and resistant to biological attack (*9*). Hemicellulose is a branched heteropolymer composed of hexose sugars (glucose, mannose and galactose), pentose sugars (xylose and arabinose) and acids (glucuronic and acetic acids) (*8*). Xylose is the predominant sugar in hemicellulose and may comprise up to 25% of the dry weight in some biomass (*8*, *10*).

In the bioethanol industry, fermentation is typically conducted with the baker's yeast *Saccharomyces cerevisiae*. This yeast utilizes hexose sugars in lignocellulosic substrates, but is unable to metabolize pentose sugars, despite the presence of a xylose transporter and all the enzymes needed for a full xylose-metabolic pathway (*11*). Both pentose and hexose sugars in potential lignocellulosic hydrolysates must be efficiently converted to ethanol to maximize the economic feasibility of a commercial bioconversion process.

Native pentose-fermenting yeasts such as *Candida shehatae*, *Candida tropicalis*, *Pachysolen tannophilus* and *Scheffersomyces* (*Pichia) stipitis* can utilize the dominant pentose and hexose sugars and convert them to ethanol. However, when presented with lignocellulosic hydrolysates, this conversion becomes inefficient for several reasons. First, native pentose-fermenting yeasts suffer from hexose repression and inactivation (*10*, *12*). When glucose and xylose are present, preferential utilization of hexose sugars occurs and pentose

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metabolism is inhibited (*10*, *13*). Second, they convert little substrate to ethanol in the presence of inhibitors arising from the pretreatment of lignocellulosic substrates, thereby limiting their fermentation performance (*14*). Third, they suffer from low ethanol tolerance which limits the amounts of ethanol that can be accumulated in the medium (*15*). To illustrate the problem, in a study of spent sulfite liquor (SSL) fermentation (*16*), *S. cerevisiae* was found to produce a greater ethanol yield (0.38 g/g total sugar) compared to *Pachysolen tannophilus* (0.12 g/g total sugar), *Pichia stipitis* (0.17 g/g total sugar) and *Candida tropicalis* (0.21g/g total sugar), even though the latter three ferment both pentose and hexose sugars. Thus, there is considerable scope for improving of the ability of the native pentose-fermenting yeasts to ferment potential lignocellulosic substrates.

Inhibitors Formed from Lignocellulosic Substrate Pretreatment

To yield fermentable sugars, lignocellulosic substrates are subjected to a pretreatment process which beak apart the lignin, hemicellulose and cellulose matrix. Examples of pretreatment processes include acid hydrolysis, alkaline hydrolysis, steam explosion (autohydrolysis), hydrothermal treatments, organosolv, ammonia freeze explosion (AFEX), ozonolysis, wet oxidation (WO) and carbon dioxide explosion processes.

Table I. Composition of selected lignocellulosic biomass

Lignocellulosic Material	*Cellulose (%)*	*Hemicellulose (%)*	*Lignin (%)*	*Reference*
Hardwood	41-50	11-33	19-30	(*17*)
Birch	40	39	21	(*18*)
Apsen	51	29	16	
Softwood	39-53	19-36	17-24	(*17*)
Spruce	43	26	29	(*18*)
Herbaceous plants	24-50	12-38	6-29	(*17*)
Switchgrass	45	30	12	(*19*)
Agricultural residues				
Corn stover	40	25	17	(*19*)
Wheat straw	30	50	20	

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A number of inhibitory compounds are formed in lignocellulosic hydrolysates from the harsh pretreatment processes. The main inhibitors include furan derivatives, organic acids and phenolics. These compounds adversely affect the viability, growth, and fermentative ability of yeasts (*14*, *17*), thereby limiting the efficiency and economic feasibility of the bioconversion process. Generally, native pentose-fermenting yeasts are more sensitive to inhibitors than *S. cerevisiae* (*18*). The reason(s) for this is not known.

Acetic acid is a weak acid released from the acetyl groups of acetylated xylan (*20*, *21*). A concentration of 5 g/L can be inhibitory to *S. stipitis* and *P. tannophilus* (*22*) and hydrolysates may contain higher concentrations. For example, hydrolysates of sugarcane bagasse and corn stover may contain 10.4 g/L (*23*) and 13 g/L (*24*) of acetic acid, respectively. During dilute acidic pretreatment, furfural and hydroxymethylfurfural (HMF) are formed from the dehydration of pentose and hexose sugars, respectively. Furfural can be further degraded to formic acid, while HMF can be broken down to levulinic and formic acids (*25*). Various phenolic compounds such as vanillin, syringaldehyde and 4-hydroxybenzaldehyde are formed from lignin degradation (*26*). The phenolics are typically found at low concentrations in hydrolysates (*27*, *28*). Nevertheless, when the inhibitors are present together, they may act synergistically, so even low concentrations of the inhibitors may contribute to the overall toxicity.

Mechanisms of Inhibition

Organic Acids

Acetic, formic and levulinic acid (Table II) are the most common weak acids found in lignocellulosic hydrolysates. The intracellular pH of yeast cells must be maintained within a physiological range for metabolic processes to proceed. The undissociated forms of these weak acids are lipid soluble and diffuse across the plasma membranes. Within yeast cells, they dissociate due to the higher intracellular pH (7.0 to 7.2), releasing protons and lowering the intracellular pH (*14*, *29*). To neutralize the intracellular pH and maintain homeostasis, the cell requires ATP to transport excess protons out *via* a plasma membrane ATPase (*29*, *30*). To generate the ATP required, sugars are utilized for energy and subsequent ethanol production and diverted from cell growth (*31*). Cytoplasmic acidification and cell death result from high levels of weak acids depleting ATP reserves, hampering cell growth and removal of protons (*32*).

The concentration of undissociated acids in hydrolysates is highly pH dependent, and a pH of at least 5.5 improves fermentation by reducing the toxicity of weak acids (*20*, *33*). Raising the pH above 5.5 in hardwood SSL can substantially reduce acetic acid toxicity by reducing the undissociated acetic acid concentration to less than 1.5 g/L (*34*).

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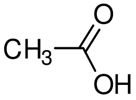
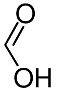
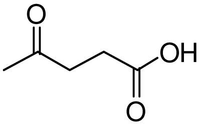
Table II. Common organic acids found in selected lignocellulosic hydrolysates

Organic acids	*pKa* *(26)*	*Chemical structure*	*Concentration in hydrolysates (g/L)*			
			Spruce *(30)*	***Olive tree cuttings*** *(35)*	***Yellow poplar*** *(36)*	***Aspen Wood*** *(37, 38)*
Acetic acid	4.75		2.4	6.8	7.1	6.1
Formic acid	3.75		1.6	2.5	ND	ND
Levulinic acid	4.66		2.6	ND	ND	ND

ND: No data available

Furan Derivatives

Furfural and HMF (Table III) are chemically related compounds containing a furan ring and aldehyde group. Furan derivatives can be metabolized by some yeast such as *S. cerevisiae* during fermentation (*39*, *40*). Furfural and HMF inhibit yeast growth and fermentation by interfering with glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase, alcohol dehydrogenase (ADH) and hexokinase. The greatest inhibitory effect of 50% was reported on purified triosephosphate dehydrogenase activity in *S. cerevisiae* by 1 g/L of furfural (*41*). Therefore, furfural adversely affects several key enzymes required for metabolism. Inhibition of ADH contributes to intracellular accumulation of acetaldehyde which at concentrations above 0.5 mM inhibit cellular functions such as DNA and protein synthesis, and can cause a lag in the growth of *S. cerevisiae* when furfural is present (*26*, *33*, *42*). In *S. cerevisiae* furfural causes reactive oxygen species to accumulate, vacuole and mitochondrial membranes damage, chromatin and actin damage (Almeida et al., 2007). Recently, Li and Juan (2010) also showed that furfural affects the biosynthesis of glycerol and metabolism of several important biochemicals at transcriptional level in *S. cerevisiae*.

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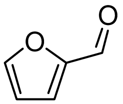
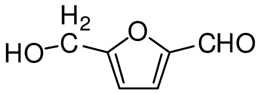
Table III. Furan derivatives found in selected lignocellulosic hydrolysates

Furan Derivatives	***Chemical structure***	***Concentration in hydrolysates (g/L)***				
		Spruce *(46)*	***Alder*** *(47)*	***Yellow poplar*** *(36)*	***Corn stover*** *(48)*	***Hardwood spent sulfite liquor*** *(49)*
Furfural		0.4	2.7-3.2	0.29	1.3-1.5	0.2
HMF		1.4	2.6-4.5	0.18	0.2	ND

ND: No data

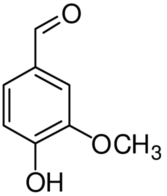
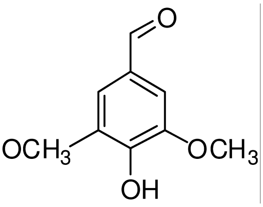
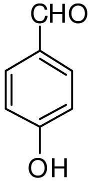
Furfural decreases the specific growth rate of *S. cerevisiae* and inhibits growth at a concentration of 5 g/L (*43*). In the presence of 2.8 g/L furfural, the cell-mass yield of *S. cerevisiae* on glucose decreased from 0.06 to 0.05 g/g (*42*). Growth of *Candida guilliermondii* was reduced by 1 g/L of furfural and 1.5 g/L of HMF (*44*). The inhibitory effects of HMF are similar to but somewhat weaker than those of furfural (*44*). Both furfural and HMF inhibited CO_2 production by *S. cerevisiae* in a synthetic medium containing 5% glucose (*45*) and presumably also ethanol production during fermentation.

Furfural can act synergistically with other inhibitors, such as acetic acid on *S. cerevisiae* (*50*) and phenols on *Escherichia coli* (*51*) to inhibit fermentation. Though concentrations of a specific inhibitor may be too low to cause adverse effects, toxicity may be enhanced by the presence of other inhibitory compounds. The growth of *S. cerevisiae* was more inhibited by 2 g/L each of HMF plus furfural than by 4 g/L of either compound alone (*52*). In another study, furfural (0.3 g/L) and HMF (0.9 g/L), when combined, decreased cell growth, ethanol production, and slowed xylose utilization in *P. tannophilus* and *S. stipitis*. The inhibitory effects were more severe with both compounds present in the medium than each individually (*14*).

Phenolic Compounds

Weakly acidic phenolic compounds listed in Table IV contain a hydroxyl group bonded to an aromatic hydrocarbon. The inhibitory mechanisms of these compounds are not well understood (*26*, *33*, *38*, *53*). Many studies investigating the toxicity of phenols have been performed using concentrations significantly higher than those present in hydrolysates (*54*). It was suggested that low MW phenolic compounds partition into the lipid bilayer of biological membranes to adversely affect membrane structure and function, and hinder the ability of membranes to act as a selective permeability barrier (*55*).

Table IV. Some common phenolic compounds found in lignocellulosic hydrolysates

Phenolic Compounds	***Chemical Structure***	***Concentration in hydrolysates (g/L)***			
		Aspen wood *(37)*	***Poplar*** *(57)*	***Red oak*** *(58)*	***Spruce*** *(59)*
Vanillin		0.23	0.003	0.09	0.096
Syringaldehyde		ND	0.002	0.213	ND
4-Hydroxybenzaldehyde		0.82	ND	ND	0.002

ND: No data available

Vanillin is formed from the degradation of the guaiacylpropane units of lignin and may be present in spruce, poplar, willow and pine hydrolysates (*17*). A low concentration of vanillin (1 g/L) was found to be toxic to *S. stipitis*, *C. shehatae*, *Zymomonas mobilis* and *S. cerevisiae*, resulting in almost complete inhibition of growth and ethanol production (*54*). Growth of *C. guilliermondii* was more severely inhibited by 1 g/L of vanillin compared to 1 g/L of syringaldehyde, and no growth occurred at 2 g/L of vanillin (*55*). The toxicity was correlated with the compound's octanol/water partition coefficient which is a measure of hydrophobicity (*56*) and suggests a membrane site of action.

Strategies To Overcome Inhibition

Physical, chemical and biological methods have been used to overcome inhibitors present in lignocellulosic hydrolysates (Table V). Many of these methods focus on removing or converting inhibitors through a detoxification step prior to fermentation. Recently, biological methods have focused on increasing yeast tolerance to inhibitors, thereby obviating the need for a detoxification

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step. It would be advantageous to not have a detoxification step as it represents a significant portion of production cost. For example, the addition of calcium hydroxide and sodium sulphite to a willow hydrolysate has been reported to account for 22% of the production costs for ethanol (*60*).

Physical Methods

Vacuum Evaporation

Vacuum evaporation is used to remove liquid and volatile compounds from samples. The procedure consists of reducing the pressure in a fluid filled container below that of the vapor pressure of the liquid, causing the liquid to evaporate along with volatile compounds. Vacuum evaporation has been used to remove inhibitors present in lignocellulosic hydrolysates (*30*, *37*). In research comparing the efficiency of alkali treatment, sulfite treatment, vacuum evaporation, anion exchange, treatment with laccase, and treatment with *Trichoderma reesei* to detoxify a dilute-acid spruce hydrolysate, vacuum evaporation was observed to be one of the least efficient methods (*30*). The spruce hydrolysate contained 5.9 g/L HMF, 1.0 g/L furfural, 2.6 g/L levulinic acid, 2.4 g/L acetic acid, 1.6 g/L formic acid, and fourteen different phenolic compounds. In a study involving vacuum evaporation, either 10 or 90% of the initial volume was removed, and the concentration of the non-volatile compounds in the hydrolysate was restored by adding water to 100% of the initial volume. Evaporation of 90% of the initial hydrolysate volume was better at eliminating inhibitors than evaporation of 10% of the initial volume. When 90% of the hydrolysate was evaporated, 65% of acetic acid, 74% of formic acid, 100% of the furfural and 4% of the HMF were removed. When 10% of the spruce hydrolysate was evaporated, only 40% of the furfural was removed. Similar results were observed in a different study examining vacuum evaporation of dilute-acid aspenwood hemicellulose containing 6.1 g/L acetic acid and 0.28 g/L furfural (*37*). In this study evaporation of the hydrolysate to near dryness at 55˚C removed all of the furfural and 46% of the acetic acid. However, vacuum evaporation did not remove the phenolic compounds. Evaporation of 90% of the hydrolysate also resulted in better fermentation results with *S. cerevisiae* than when 10% was evaporated. The ethanol yield for 90% evaporation-treated spruce hydrolysate was 0.42 g/g, compared to 0.32 g/g, and 0.34 g/g for untreated spruce hydrolysate and 10% evaporation-treated spruce hydrolysate, respectively. Vacuum evaporation, unlike some other physical detoxification methods (ie., anion exchange), does not result in a decreased sugar content (*30*). However, these results along with other studies (*61*) suggest that volatile compounds in lignocellulosic hydrolysates are not the major inhibitors. Larsson et al. (1999) pointed out the possibility of combining laccase treatment and evaporation, as the laccase enzyme would remove phenols, and evaporation would remove furfural. However, it is desirable to eliminate or reduce detoxification steps for economical industrial production of ethanol.

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Solvent Extraction

Solvent extraction of lignocellulosic hydrolysates as a detoxification method has been examined (*37*, *62*, *63*). Ethyl acetate was used to extract low MW phenolics from an enzyme pretreated hydrolysate of aspen wood prior to fermentation by *S. stipitis* (*37*). The aspen wood hydrolysate contained 20.2 g/L xylose, 2.6 g/L glucose, 5.3 g/L acetic acid, 0.05 g/L furfural, 0.82 g/L hydroxybenzoic acid and 0.21 g/L vanillin. Ethyl acetate extraction at a 1:1 (v/v) ratio of ethyl acetate to aspen wood hydrolysate reduced the acetic acid to 2.7 g/L, and completely removed the furfural, hydroxybenzoic acid, and vanillin. No ethanol was produced from fermentation of untreated aspen hydrolysate. Fermentation of ethyl acetate extracted aspen hydrolysate gave an ethanol yield of 0.47 g/g. One disadvantage of liquid extraction is the requirement of a large volume of solvent, and distillation would be required to recycle the solvent, adding to production cost and time.

Table V. Common detoxification methods to increase the fermentability of lignocellulosic hydrolysates

Method	***Description***	***Reference***
Physical Methods		
Vacuum evaporation	Evaporation of volatile inhibitors, such as furfural, acetic and formic acids.	(*30*, *37*, *61*)
Solvent extraction	Removal of phenolic compounds.	(*37*, *62*, *63*)
Ion exchange resins	Adsorb aliphatic acids, furans and phenolic compounds. Ion exchange resins are expensive.	(*30*, *46*, *64*, *65*)
Activated charcoal	Adsorb furans, phenolic compounds, and acetic acid.	(*64*, *66*, *67*)
Lignin residue	Adsorb furans and phenolic compounds.	(*68*)
Chemical Method		
Alkali treatment	Addition of an alkali base to adjust pH to 9-10, followed by readjustment of pH to 5.5. Inhibitor removal is though chemical conversion. Loss of sugars has been reported.	(*69–79*)
Biological Methods		
Bioabatement	Treat hydrolysate with microorganisms that utilize or convert inhibitors to less toxic compounds.	(*80–84*)
Laccase enzyme treatment	Removal of phenolic compounds.	(*64*, *85–88*)

Continued on next page.

Table V. (Continued). Common detoxification methods to increase the fermentability of lignocellulosic hydrolysates

Method	*Description*	*Reference*
Fed-batch fermentation	Allows *in-situ* detoxification of inhibitors by fermenting yeast by keeping inhibitor concentration low through control of substrate feed rate.	(*89–93*)
Continuous fermentation with cell retention system	*In-situ* detoxification coupled with a cell retention system to maintain high cell density. Faster fermentation times than with fed-batch.	(*94–101*)
Yeast adaptation	Utilizes yeasts adaptation ability to increase inhibitor resistance.	(*102–106*)
Recombinant yeast	Molecular techniques are used to create recombinant yeast with increased resistance to inhibitors	(*6*, *107–114*)
Evolutionary engineering of yeast	Development of inhibitor tolerant yeast through iterative genetic diversification and functional selection.	(*115*, *116*)

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Removal of Charged Inhibitors by Ion-Exchange Resins

Ion-exchange resins have been used to detoxify lignocellulosic hydrolysates (*30*, *46*, *65*). Ion-exchange involves exchanging an ion from solution for a similarly charged ion attached to an immobile solid particle. Both cation and anion exchange resins have been used to remove inhibitors in hydrolysates. In one study, a dilute-acid spruce hydrolysate containing 5.9 g/L HMF, 1.0 g/L furfural, 2.4 g/L acetic acid, 2.6 g/L levulinic acid, 1.6 g/L formic acid, and 14 different phenolic compounds was treated with polystyrenedivinylbenzene-based anion-exchange resins at either pH 5.5 or 10 for 1 h in a batch procedure (*30*). The anion exchange resins were effective at removing the aliphatic acids, furan derivatives and phenolic compounds from the spruce hydrolysate. Anion exchange at pH 10 removed 70% of the HMF, 73% of the furfural, 97% of the formic acid, 96% of the acetic acid, and 93% of the levulinic acid from the spruce hydrolysate. The spruce hydrolysate contained 32.3 g/L of fermentable sugars. After treatment with anion exchange the hydrolysate was fermented with *S. cerevisiae*, resulting in an improved ethanol yield (0.49 g/g), volumetric productivity (1.42 g/L/h) and biomass yield (0.08 g/g) in comparison to the untreated hydrolysate's ethanol yield (0.32 g/g), volumetric productivity (0.04 g/L/h) and biomass yield (0.01 g/g). Although, anion exchange at pH 10 was effective at removing inhibitors and improving hydrolysate fermentation, it resulted in a 26% decrease in sugar content. However, other studies using hydrolysates pretreated with anion exchange resins did not report any decrease in sugar content (*46*, *64*, *65*).

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The presence of counter ions, such as sulfate, was found to be important in preventing sugar loss when using ion exchange (*65*). Anion exchangers were found to have the ability to trap glucose monomers, but the addition of sulfate ions resulted in no significant loss of sugars (*65*).

A comparison of a cation exchanger, anion exchanger, and a resin without charged groups at either pH 5.5 or 10 was done to determine which was most effective at removing inhibitors from a dilute-acid spruce hydrolysate (*65*) which contained 7.97 g/L aliphatic acids, 5.10 g/L HMF, 0.82 g/L furfural and 3.7 g/L phenolic compounds. Treatment with an anion exchanger at pH 10 was found to be the most effective at removing phenolic compounds, furan aldehydes and aliphatic acids. Anion exchange at pH 10 removed 96% of the aliphatic acids, 65% of the HMF, 68% of the furfural, and 81% of the phenolic compounds. For all three types of resins, greater improvement in fermentation was seen when treatment was conducted at pH 10 rather than pH 5.5. Fermentation of spruce hydrolysate treated by anion exchange resins at pH 10 by *S. cerevisiae* resulted in an ethanol yield of 0.46 g/g, compared to 0.06 g/g for the untreated hydrolysate (*65*).

Horvath et al. (2004) reported that treatment with anion exchange resins made from styrene based matrices containing strongly basic functional groups improved fermentation of dilute-acid spruce hydrolysates and improved ethanol production by 7-fold compared to fermentation of untreated hydrolysates. The hydrolysate originally contained 15.7 g/L glucose, 12.8 g/L mannose, 1.4 g/L HMF, 0.4 g/L furfural, 1.4 g/L levulinic acid, 3.2 g/L acetic acid, 1.1 g/L formic acid, and 2.4 g/L phenolic compounds. Treatment with the anion exchange resins made from a styrene based matrix containing strongly basic functional groups removed 90% of the levulinic acid, 72% of the acetic acid, 73% of the formic acid, 38% of the furfural and 29% of the HMF. *S. cerevisiae* was used to ferment the hydrolysate. Treatment with resins containing weak basic functional groups led to the least improvement in ethanol productivity from fermentation of treated hydrolysate. Ion exchange resins represent an efficient method to detoxify lignocellulosic hydrolysates, and result in improved fermentation and ethanol yields. However, the use of ion exchange resins is costly and may be uneconomical and impractical for large-scale ethanol production.

Removal of Inhibitors by Activated Charcoal

A few studies have examined the use of activated charcoal or carbon to remove inhibitors from lignocellulosic hydrolysates. Activated carbons are less expensive than ion exchange resins and can be regenerated using steam (*66*). Activated carbon has been successfully used to reduce acetic acid concentration from 10 to 4 g/L in a synthetic hydrolysate shaken at 250 rpm (*67*). However, activated carbon was found to be marginally successful at removing acetic acid from dilute acid corn stover hydrolysate (*66*). Five rounds of activated carbon adsorption were required to bring acetic acid levels from an initial concentration of 16.5 g/L to below 2 g/L. Negligible amount of glucose (<1 g/L) was lost after activated carbon treatment. Xylose losses were not measured.

In another study, a dilute-acid sugarcane bagasse hydrolysate containing 1.89 g/L furans, 2.75 g/L phenolic compounds and 5.45 g/L acetic acid was treated with activated charcoal, resulting in 38.7, 57, and 46.8% reductions in furans, phenolics, and acetic acid, respectively (*64*). Fermentation of the activated charcoal treated hydrolysate by *C. shehatae* led to a maximum ethanol concentration of 7.43 g/L, and an ethanol yield of 0.425 g/g. Fermentation of untreated bagasse hydrolysate was not done, but in comparison, neutralization, which did not remove inhibitors to any appreciable level, resulted in an ethanol yield of 0.22 g/g. The study did not report if the sugar content had decreased after treatment with activated charcoal. From an economic standpoint it is important that a detoxification procedure results in minimal if any sugar loss, and does not require multiple rounds of application.

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Treatment with Lignin Residues

Limited research was undertaken using lignin residues to remove the inhibitors in lignocellulosic hydrolysates. Lignin is relatively inexpensive and abundant. After acid hydrolysis of lignocellulose, the solid phase lignin can be dried and used for detoxification purposes. Lignin has hydrophobic properties that have been used to detoxify dilute-acid spruce hydrolysates with limited success (*68*). The detoxification effect of the lignin was improved when a large amount of water-washed lignin was used during low treatment temperatures. The spruce hydrolysate contained 38.3 g/L of fermentable sugars, 2.0 g/L HMF, 0.6 g/L furfural, and 3.3 g/L phenolic compounds. The spruce hydrolysate was treated with 100 g/L of lignin (obtained from the hydrolysis of spruce) at pH 5.5 and 4˚C. The procedure removed 27% of HMF, 49% of furfural, and 36% of phenolic compounds. Fermentation with *S. cerevisiae* of the spruce hydrolysate treated with 100 g/L of lignin at pH 5.5 resulted in an ethanol yield of 0.44 g/g. This was a significant improvement compared to the ethanol yield of 0.08 g/g from fermentation of the untreated hydrolysate. The ethanol yield, 0.44 g/g of the treated hydrolysate was close to the ethanol yield, 0.46 g/g, of the reference fermentation, which contained 35 g/L of glucose in a defined medium. The authors suggested that more effort should be directed at increasing the number of interaction sites in lignin to improve its adsorptive capabilities for better inhibitor removal.

Chemical Method

Alkali Treatment

Alkali treatment is a well known chemical method used for detoxifying lignocellulosic hydrolysates. Alkali treatment involves adjusting the pH to 9-10 through the addition of an alkali base (eg., $Ca(OH)_2$, NaOH, KOH, NH_4OH)) followed by readjustment of the pH to 5.5 for fermentation (*72*, *78*). Although detoxification of hydrolysates with alkali treatment has been used for many years, little is understood about the chemical mechanism(s) behind the detoxification process, and what influence the cation (Ca, Na, K) have on subsequent fermentation. Based upon analyses of the solid residues formed after alkali treatment, detoxification likely occurs through chemical conversion of the inhibitors rather than through precipitation and filtration (*77*). Supercritical fluid extraction (SFE) was used to extract inhibitors from the precipitate after alkali treatment to determine whether inhibitors were removed via adsorption to the precipitate or rather through conversion of the individual inhibitors (*77*). HPLC analysis of the SFE extracts from the precipitate revealed the presence of HMF, 2-furaldehyde, 4-hydroxybenzoic acid, vanillic acid, syringic acid, R-hydroxyguaiacone, phenol, vanillin, coniferyl alcohol, and coniferyl aldehyde. However, the inhibitors were only present in amounts that were less than 1% of the original amount present in the hydrolysate. This suggested precipitation and filtration were not responsible for the detoxification effects of alkali treatment. Optimal alkali treatment conditions for detoxification are variable and depend upon the mineral acid and organic acid composition of the hydrolysate (*74*).

Overliming is the addition of calcium hydroxide to hydrolysates. It is inexpensive yet an effective detoxification method. Overliming produces a precipitate containing calcium salt that must be removed from the mixture. Heat is sometimes used in the overliming process because calcium sulfate, the main salt in the precipitate, has decreased solubility at high temperatures, and volatile inhibitors can be evaporated out of the hydrolysate solution (*76*). Since hydrolysates emerge from the dilute acid pretreatment step at or near 100°C, the application of overliming treatment at a high temperature would obviate the need for cooling of the hydrolysate (*70*). More inhibitors, such as furfural and HMF are removed from hydrolysates when overlimed at higher temperatures (*73*, *74*). However, greater sugar losses have been observed when overliming at higher temperatures (*73–75*). In one study, overliming a dilute acid spruce hydrolysate using $Ca(OH)_2$ led to greater sugar losses at higher temperatures than at lower temperatures (*75*). Here, overliming of the spruce hydrolysate at 60°C and pH 10 resulted in decreases of 12% arabinose, 17% galactose and glucose, 19% mannose, and 23% xylose. On the other hand, overliming at 25°C and pH 10 did not result in any decrease in the concentration of glucose, mannose, xylose, and only a 1% decrease in arabinose and galactose.

The influence of the cation of the alkali base on the effectiveness of detoxification was assessed using a dilute-acid spruce hydrolysate and a less complex synthetic inhibitor cocktail (*77*). The ability of calcium hydroxide, sodium hydroxide, potassium hydroxide, and ammonia to detoxify the spruce hydrolysate was compared. Alkali treatment of the spruce and synthetic hydrolysate was carried out at room temperature for one hour. The concentration of the main inhibitors in the spruce hydrolysate were 2.4 g/L HMF, 0.7 g/L furfural, 3.1 ppm 3,4-dihydroxybenzaldehyde, 1.9 ppm 4-hydroxybensaldehyde, and 1.1 ppm cinnamic acid. In general, all treatments resulted in a decrease in HMF, furfural, 3,4-dihydroxybenzaldehyde, 4-hydroxybenzaldehyde, and cinnamic acid concentrations, with greater decreases found at pH 10 than at 5.5. For example, treatment with calcium hydroxide and ammonia resulted in improved removal of furaldehydes than treatment with sodium and potassium hydroxide. Also, calcium hydroxide and ammonia were more effective at removing HMF than sodium and potassium hydroxide. Calcium hydroxide and ammonia removed 30-40% of HMF, compared to 20% by sodium and potassium hydroxide. The concentration

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of 3,4-hydroxybenzaldehyde decreased by 40-60% when treated at pH 10 for all treatments. All treatments reduced 4-hydroxybenzaldehyde by 30-40%, except for ammonia, which only resulted in a 10% decrease. Cinnamic acid was reduced by 80% by calcium hydroxide and ammonia, and 40% by sodium and potassium hydroxide treatment. Fermentation of the treated hydrolysates supplemented with 1 g/L yeast extract, 0.5 g/L $(NH_4)_2HPO_4$, 0.025 g/L $MgSO_4{\circ}7H_2O$, and 1.38 g/L NaH_2PO_4 was carried out at pH 5.5 using *S. cerevisiae*. In general, greater ethanol production was observed for samples that had undergone alkali treatment at pH 10 than at pH 5.5. For example, treatment with calcium hydroxide at pH 10 led to an ethanol yield of 0.46 g/g and a volumetric productivity of 1.92 g/L/h during the first 7 hours of fermentation. Treatment with calcium hydroxide at pH 5.5 gave an ethanol yield of 0.37 g/g and a volumetric productivity of 0.33 g/L/h. Untreated hydrolysate gave an ethanol yield of 0.35 g/g. The greatest volumetric productivity was attained in hydrolysates treated by calcium hydroxide, followed by ammonia, and finally sodium hydroxide. The mechanism(s) responsible for the improved fermentability of the hydrolysates after calcium hydroxide treatment is not known.

Alkali treatment is an inexpensive and effective detoxification method; however it results in an undesirable decrease in sugar content, which reduces the final ethanol yield (*75*, *77*). For example, detoxification of dilute-acid treated spruce hydrolysate by NaOH at pH 9 at 80˚C for one hour resulted in a 25% decrease of glucose from an initial concentration of 21.9 g/L (*75*). In comparison, treatment with calcium hydroxide under the same conditions resulted in a 40% loss of glucose. As the pH, temperature and exposure time increased, the amount of sugar loss also increased, but a greater amount of furfural and HMF were removed also. The initial concentrations of acetic acid (3.1 g/L) and formic acid (0.9 g/L) were increased 2-fold when the hydrolysate was subjected to severe treatment conditions at 80˚C and pH 12. Decreases in sugar levels of up to 8.7% have been reported when a dilute-acid hydrolysate of bagasse was overlimed at 60˚C (*74*). To prevent sugar degradation and formation of excessive amounts of potentially inhibitory aliphatic acids, it has been suggested that alkali treatment conditions should be within a pH range of 9-12 at temperatures under 30˚C (*75*).

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Biological Methods

Bioabatement of Inhibitors in Lignocellulosic Hydrolysates

Bioabatement is method that utilizes microorganisms to detoxify inhibitors present in lignocellulosic hydrolysates. Ideally bioabatement results in little formation of waste, does not affect the sugar content, is inexpensive and can be carried out directly in the vessel used for fermentation (*117*). Microorganisms used for bioabatement of hydrolysates reduce toxicity by utilizing inhibitors or by converting them to less toxic compounds.

Lopez et al. (2004) isolated the fungus *Coniochaeta ligniaria* NRRL 30616 from furfural contaminated soil, and used it to detoxify a dilute-acid pretreated corn stover hydrolysate (CSH). After 30 hours of incubation with *C. ligniaria*, 78% of HMF and 97% of furfural was eliminated from CSH. *C. ligniaria* converts furfural to furfuryl acid and furoic aicd and HMF to 2,5-bis-hydroxymethylfuran (*82*).

C. ligniaria treatment has been tested for its ability to aid in the utilization of xylose in CSH by *S. cerevisiae* LNH-STN (*82*). In this experiment cellulase enzymes were not added to keep the glucose concentrations low (less than 2.5 g/L). The initial xylose concentration of the CSH was 1.1% (w/v). The CSH was incubated with *C. ligniaria* for 24 hours prior to fermentation by LNH-STN. After 20 hours of incubation of abated CSH with LNH-STN, xylose in the CSH was reduced to levels below 0.2% (w/v). In the unabated CSH, it took 120 hours for xylose levels to reach 0.2% (w/v).

One negative aspect of bioabatement is if the detoxifying microorganism metabolizes some of the sugars in the hydrolysate, this lowers the sugar concentration available for fermentation and reduces the final ethanol yield. Nichols et al. (2005) reported that in addition to lowering furfural, HMF and acetate content, *C. ligniaria* reduced glucose levels from 0.35 to 0.19% and xylose levels from 1.81 to 1.47% in the CSH. The utilization of sugars by bioabatement microorganisms can be addressed by developing mutants of detoxifying microorganisms unable to metabolize the sugars. One such detoxifying yeast mutant that removes acetic acid from hardwood spent sulfite liquor (HW SSL), while not significantly lowering the sugar concentration of the hydrolysate, is *S. cerevisiae* YGSCD 308.3 (*84*), which utilizes acetic acid but not hexose or pentose sugars. After 24 hours incubation of strain YGSCD 308.3 with steam stripped HW SSL, the acetic acid concentration was lowered from 0.68 to less than 0.04% (w/v) (*84*). After significant reduction in the acetic acid concentration, YGSCD 308.3 treated HW SSL supported better growth of *Pachysolen tannophilus* and *S. stipitis* and improved fermentation compared to untreated HW SSL. Mutations in the genes encoding kinases specific to phosphorylation of glucose and mannose render the yeast mutant unable to metabolize these sugars (*118*, *119*). In addition, the yeast is unable to use xylose or arabinose. The mutation in the gene for hexokinase II allows this yeast to utilize carbon sources, such as acetic acid in the presence of the preferred substrate glucose. A normal hexokinase II in the presence of glucose would repress other enzymes that are responsible for metabolizing other carbon sources (*84*).

Recently, a thermophilic bacterium *Ureibacillus thermosphaericus* was examined for bioabatement of the inhibitors in waste house wood (WHW) (*83*). *U. thermosphaericus* is of particular interest because it is fast growing and does not utilize sugars. Its use in bioabatement will not affect the fermentable sugar concentration in the hydrolysate. The original total sugar concentration in WHW was 50.2 g/L, and after treatment with *U. thermosphaericus* sugar loss was less than 5%. The author suggested the sugar loss was due to decomposition of sugars by extracellular oxidases because *U. thermosphaericus* does not assimilate sugars. After incubation of WHW with *U. thermosphaericus* at 50 ºC for 24 hours, the ethanol production rate by *S. cerevisiae* was 12.5 g/L/h, which was much higher than that (2.5 g/L/h) with the untreated WHW (*83*). A solution containing 1.0 g/L furfural and another solution containing 1.5 g/L of HMF were treated with *U. thermosphaericus* at 50 °C for 24 h. The bacterium oxidized furfural to 2-furancarboxylic acid and HMF to 5-hydroxymethyl furancarboxylic

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acid, compounds that are less toxic to fermentative yeasts. The WHW contained 0.9 g/L of HMF and 0.5 g/L of furfural. Interestingly, *U. thermosphaericus* did not convert HMF in the WHW as effectively as it did with the HMF solution. For example, the HMF concentration was decreased by 50% in the HMF solution, but only by 20% in the WHW. Okuda et al. (2008) suggested that a decrease in HMF conversion in the WHW may be due to inhibition or repression extracellular oxidases secreted by *U. thermosphaericus*.

Bioabatement may not yet represent a viable detoxification method for cost effective ethanol production on an industrial scale. The incubation time needed for bioabatement would add significantly to the process time, and this may not be economically feasible (*81*).

Treatment with Laccase

The use of enzymes to detoxify inhibitors from lignocellulosic hydrolysates has been examined (*85–88*). Enzymes tested for this purpose have been isolated mostly from ligninolytic white rot fungi, with a primary focus on laccases, copper-containing phenol oxidases (*85*, *87*, *88*). Laccase oxidizes phenols and aromatic amines in lignin materials to form water and unstable radicals that form high molecular mass polymerization products, resulting in removal of low MW phenolic compounds from the hydrolysate (*113*).

After treatment with laccase, lignocellulosic hydrolysates have shown improved fermentability (*87*, *88*). The fermentation of steam and acid pretreated willow hydrolysate detoxified by laccase has been evaluated (*87*). The hydrolysate was incubated at 30°C for 12 hours with 1 μM of laccase and the laccase-treated hydrolysate was then fermented for 9 hours with *S. cerevisiae*. Compared to untreated hydrolysate, fermentation of the laccase treated hydrolysate resulted in improved volumetric ethanol productivity (2.7 vs. 0.8 g/L/h), and glucose consumption (4.8 vs. 1.5 g/L/h) (*87*). However, the ethanol yield was not greatly improved over the untreated hydrolysate, 0.55 vs 0.53 g/g, respectively. It may be possible that the laccase enzyme may have served as a nutrient for the yeast during fermentation, leading to a better fermentation performance. Treatment with 1 μM of laccase resulted in a decrease in phenol concentration of the willow hydrolysate from 2.5 to 0.16 g/L. In other research, fermentation of laccase-treated acid pretreated bagasse hydrolysate by *C. shehatae* resulted in an ethanol yield of 0.374 g/g (*64*). Fermentation of an untreated bagasse hydrolysate was not done. Fermentation of a neutralized hydrolysate gave an ethanol yield of 0.22 g/g.

Jurado et al. (2009) studied the effects of harsh vs. soft pretreatment of hydrolysates on the efficacy of laccase detoxification. Wheat straw was either impregnated with sulfuric acid (harsh pretreatment) or water (soft pretreatment) before steam explosion and was followed with treatment with laccase. The hydrolysates were treated for two hours at pH 5 with 0.5 U mL^{-1} of laccase. Laccase treatment reduced phenolic content in water impregnated steam exploded wheat straw from 0.58 to 0.15 g/L. Treatment of acid impregnated straw with laccase reduced the phenolic content from 0.198 to 0.058 g/L. Laccase treatment improved fermentation of the wheat straw hydrolysate for both types of

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pretreatment. For water pretreated hydrolysates, removal of phenolic compounds resulted in an ethanol yield 2.7 times higher than the control hydrolysate not treated with laccase. Acid pretreated wheat straw hydrolysate that was treated with laccase had ethanol yields 2 times higher than those not treated with laccase.

Although the more severe acid pretreatment condition results in a greater sugar content than the less harsh water pretreatment condition, the generation of a greater concentration of inhibitors, such as furfural and HMF is more likely (*120*, *121*). The acid pretreated hydrolysate may have had inhibitors present that were unaffected by laccase treatment and the difference in improvement of ethanol productivity may be due to differences in inhibitor concentration between the acid and water pretreated hydrolysates. Notably, Chandel et al. (2007) found similar results when comparing laccase detoxification of acid pretreated bagasse hydrolysate to detoxification by ion-exchange resins. A higher ethanol yield was achieved with ion-exchange resins, which removed phenols, furans and acids, than with laccase, which only removed phenols. These findings reinforce the importance of understanding the severity of pretreatment conditions when selecting a detoxification method.

Manipulating Fermentation Conditions

Fermentation of hydrolysates can be performed in batch, fed-batch, or continuous mode. The design of a fermentation process should have low equipment cost and result in a high ethanol yield and productivity. Traditional batch fermentations of lignocellulosic hydrolysates usually generate dismal results without a prior detoxification step (*90*, *122*). The concentration of hydrolysate inhibitors in batch fermentations may be above a threshold for fermenting yeasts to deal with (*90*, *123*, *124*). Different modes of fermentation have been used to obviate the need for a detoxification step (*89*, *93*, *95*, *99*, *100*).

Fed-Batch Fermentation

Fed-batch fermentation can be used to prevent high concentrations of inhibitors as the feed rate of hydrolysate into the reactor can be controlled. In one study, fermentation of a dilute-acid spruce hydrolysate by *S. cerevisiae* was possible using the fed-batch mode, while batch fermentation was unsuccessful (*93*). The spruce hydrolysate contained 2.2 g/L furfural and 7.2 g/L HMF. The selection of the optimal substrate feed rate was critical in successful fed-batch fermentation. The optimal substrate feed rate depends on the composition of the hydrolysate and the capacity of the yeast to convert inhibitors (*90*, *93*). In this study, ethanol yields of 0.43 g/g were achieved for feed rates of 25 and 50 mL/h, while no ethanol was produced with a 100 mL/h feed rate. Likewise, ethanol was not produced during batch fermentation. During fed-batch fermentation 90% of the furfural and 40-70% of the HMF were converted (*93*). The spruce hydrolysate had an initial concentration of 39 g/L glucose, 12.3 g/L mannose and 3.3 g/L xylose. It is noted that one important disadvantage of fed-batch fermentation is the longer time required.

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Continuous Fermentation and Cell Retention

Continuous fermentation systems employing high densities of yeasts have been used for the fermentation of lignocellulosic hydrolysates (*94*, *96–100*). Continuous fermentation has been used to reduce fermentation time, and reduce production costs (*99*). To keep the concentration of inhibitors low, substrate is fed at a low rate in continuous mode, similar to the fed-batch mode. The key requirement of continuous fermentation is a cell growth rate equal to the dilution rate to prevent washout of the cells. However, low dilution rates, coupled with decreased growth due to the presence of inhibitors have resulted in low ethanol productivity of continuous fermentation of lignocellulosic hydrolysates (*97*, *98*). High cell density in the fermentor can be maintained by using cell retention systems, which typically leads to increased ethanol productivity. Cell recycling (*98*, *125*), immobilization (*95*, *99*), encapsulation (*101*)and flocculating yeasts (*100*) have been used to maintain high cell density.

Good fermentation results have been obtained with the recycling of a flocculating *S. cerevisiae* strain when using a dilute-acid hydrolysate as a substrate (*100*). Flocculation allows easy separation in a settler with less mechanical stress, resulting in high cell retention (*100*). High substrate dilution rates of up to 0.52 h^{-1} can be used, resulting in ethanol yields in the range of 0.42-0.46 g/g. The ability of flocculating yeast to rapidly re-flocculate after being separated and settle to the bottom allows the use of simple separation equipment for cell recycling. This in turn reduces the operating costs substantially (*100*). However, flocculating yeast takes twice the amount of time to complete fermentation as compared to free cells. This is a disadvantage to efficient ethanol production.

Cells of *S. cerevisiae* CBS 8066 immobilized in alginate beads have been used with continuous stirred tank (CSTBR) and fluidized bed bioreactors (FBBR) in series to ferment an undetoxified dilute acid spruce hydrolysate (*99*). The use of a CSTBR and FBBR in series reduced the problem of sugars washing out due to low sugar assimilation by immobilized yeast cells when substrate feed rates are high. A single CSTBR was able to assimilate up to 75% of the initial glucose concentration of 20 g/L in the spruce hydrolysate at a dilution rate of 0.43 h^{-1}, and up to 54% of the sugars at a dilution rate of 0.86 h^{-1}. Adding a FBBR in series to the CSTBR resulted in 100 and 86% assimilation of sugars at dilution rates of 0.43 and 0.86 h^{-1}, respectively. Good ethanol yields from the spruce hydrolysate were achieved for all of the experiments: 0.41 to 0.48 g/g. The single CSTBR resulted in 96.2% furfural conversion and a 41.4% HMF conversion at a dilution rate of 0.86 h^{-1}. Improved furfural (100%) and HMF (75.3%) conversions were observed when using the serial bioreactor with a dilution rate of 0.86 h^{-1}.

A dilute acid spruce hydrolysate has been fermented to ethanol by an alginate-encapsulated *S. cerevisiae* using continuous fermentation at dilution rates up to 0.5 h^{-1} (*101*). The encapsulated cells showed improved in situ detoxification and were able to ferment the hexose sugars in the hydrolysate at higher feed rates, 0.1-0.5 h^{-1}, while maintaining at least 75% viability. In contrast, the freely suspended cells could only ferment the hydrolysate continuously up to a dilution rate of 0.1 h^{-1}, after which 75% of their viability was lost. The encapsulated cells were able to produce good ethanol yields of 0.44 g/g at all feed rates, with specific productivities of 0.14-0.17 g/g/h. Encapsulation provides a higher cell concentration in the bioreactor than free cells, resulting in a higher volumetric productivity (*101*). Also, separation of the biomass from the medium is easier with encapsulated than free yeast cells.

Yeast Adaptation for Improved Inhibitor Tolerance

Adaptation of fermenting yeasts to toxic compounds in hydrolysates is a low-cost technique to overcome inhibition from toxic compounds in lignocellulosic hydrolysates. This approach reduces reliance on more expensive detoxifying steps, thereby reducing the cost and time required to produce ethanol from lignocellulosic hydrolysates. Adaptation can decrease fermentation time and increase yeast tolerance to inhibitors present in hydrolysates. Adaptation of yeast cells to inhibitors can be done by growing the strain in a dilution of hydrolysate, cultivating the yeast in increasing concentrations of hydrolysate, or cultivating the yeast on the hydrolysate before fermentation (*102*, *103*, *105*).

Recombinant xylose-fermenting *S. cerevisiae* TMB 3001 was adapted to inhibitors present in sugarcane bagasse hydrolysate by growing the yeast in a medium containing hydrolysates with increasing concentrations of toxic inhibitors (*105*). This approach led to an adapted yeast strain that had better ethanol yields and productivity compared to the original unadapted yeast strain. After a 24 hour fermentation of 50% sugarcane bagasse hydrolysate containing 25.8 g/L glucose and 5.9 g/L xylose, the adapted yeast strain gave an ethanol yield of 0.38 g/g, while the unadapted strain gave an ethanol yield of 0.18 g/g. Also, the adapted strain had an ethanol productivity of 2.55 g/g of initial biomass per hour, while the unadapted strain had an ethanol productivity of 1.15 g/g/h.

The adapted yeast converted inhibitors in the hydrolysate faster than the non-adapted yeast. With the 50% bagasse hydrolysate, both yeast strains converted furfural to furfural alcohol within 24 hours, but the adapted yeast had a maximal conversion rate of 0.150 g/L/h, while the unadapted yeast achieved a rate of 0.075 g/L/h, only half that of the adapted yeast (*105*). Likewise, in 75% bagasse hydrolysate with a total furan concentration of 3.8 g/L, the adapted strain converted 74% of the initial furfural and 40% of the initial HMF, whereas the unadapted strain converted 22% of the initial furfural and 20% of the initial HMF (*105*).

Other researchers have found success in adapting yeasts to inhibitors present in lignocellulosic hydrolysates by growing yeast strains in media with increasing concentrations of hydrolysate. *S. cerevisiae* D_5A that was gradually adapted to softwood hydrolysate produced 2.3 times more ethanol than the unadapted parent strain when grown with 6.5% (w/w) dry basis of hydrolysate with an initial glucose concentration of 10.85 g/L (*104*). Successful adaptation of *S. stipitis* to inhibitors present in corn cob hydrolysate was achieved by repeatedly subculturing the yeast in media containing increasing concentrations of hydrolysate (*103*). Fermentation of $Ca(OH)_2$ treated corn cob hydrolysate by adapted *S. stipitis* resulted in improved ethanol yields of 0.41 g/g compared to that (0.32 g/g) achieved by the unadapted yeast. The adapted *S. stipitis* strain attained 82% of the theoretical ethanol yield,

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which is similar to that reported by Parekh et al. (1986) with *S. stipitis* adapted to wood hydrolysate (*103*).

Adaptation of *Candida shehatae* and *S. stipitis* to aspen wood hydrolysate was accomplished by recycling the yeast cells 72 and 12 times, respectively, in cultures containing aspen wood hydrolysate (*106*). Fermentation of aspen wood hydrolysate by adapted *S. stipitis* resulted in utilization of 95% of the 92 g/L of glucose and xylose in the hydrolysate, and an ethanol yield of 0.47 g/g (*106*). This is improved over the results of the unadapted *S. stipitis*, which utilized 78% of the sugars, and had an ethanol yield of 0.41 g/g. Adapted *C. shehatae* had a slightly improved ethanol yield of 0.41 g/g over that of unadapted *C. shehatae* (0.39 g/g).

Adaptation experiments with co-cultures of *S. cerevisiae* 2.535 with either *Pachysolen tannophilus* ATCC 2.1662 or a recombinant *E. coli* yielded some interesting findings (*122*). After 5 rounds of growth on soft wood hydrolysate the co-cultures were examined under a microscope. The adapted *S. cerevisiae* represented a greater proportion of the cells in the media, and were larger than the other cells. Interestingly, the adapted *S. cerevisiae* cells were clustered together with *P. tannophilus* or recombinant *E. coli* cells surrounded by or located within the cluster. The co-culture of cells may be aggregating together in clusters because of a lowered local concentration of inhibitors due to adapted *S. cerevisiae* detoxifying the inhibitors present in the hydrolysate (*122*). Improved ethanol yields were achieved by the adapted co-cultures compared to the unadapted co-cultures. The adapted co-culture of *S. cerevisiae* and *P. tannophilus* gave an ethanol yield of 0.49 g/g after fermentation of NaOH treated softwood hydrolysate with an initial sugar concentration of 37.5 g/L, whereas the unadapted co-culture gave an ethanol yield of 0.26 g/g. Likewise, the ethanol yield from fermentation of NaOH treated hydrolysate for the adapted co-culture of *S. cerevisiae* and recombinant *E. coli* was better than the unadapted co-culture, 0.45 g/g compared to 0.24 g/g. The adapted co-culture of *S. cerevisiae* and recombinant *E. coli* had a much better ethanol yield from fermentation of untreated hydrolysate than the adapted co-culture of *S. cerevisiae* and *P. tannophilus*, 0.34 g/g and 0.16 g/g, respectively.

How yeast cells become acclimatized to the inhibitors present in hydrolysates is not known. It is hypothesized that growth in the presence of inhibitors modifies gene expression, leaving the yeast "primed" for future exposure to inhibitors. For instance, yeast cells grown on hydrolysate is likely to have a different set of genes being expressed compared to yeast cells grown on glucose (*102*). The generation of mutants occurring during adaptation is possible, but limited due to small number of generations occurring during adaptation. This again places emphasis on the potential of different gene expression profiles for adapted yeast compared to unadapted yeasts. The adaptation of fermenting microbes that already have substantial tolerance to inhibitors could be especially useful to develop microorganisms with even higher inhibitor tolerance for ethanol production from lignocellulosic hydrolysates.

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Recombinant Yeasts with Improved Inhibitor Tolerance

The advancement in genetically engineered yeasts with greater inhibitor tolerance is a promising option to the traditional costly detoxification steps. One way to improve yeast tolerance to inhibitors is to create recombinant yeasts expressing enzymes that confer resistance to specific inhibitors.

Improved Furfural and HMF Tolerance

Some researchers have created yeast strains that are more efficient at reducing furfural and HMF to their corresponding alcohols, 2-furanmethanol (*102*)and 2,5-bis-hydroxymethylfuran, which are significantly less toxic (*102*). *S. cerevisiae* TMB3000 is naturally resistant to inhibitors present in spruce hydrolysates (*126*). TMB3000 has increased resistance to furfural and is more efficient at metabolizing furfural compared to *S. cerevisiae* CBS8066, a laboratory strain (*127*, *128*). Correspondingly, furfural and HMF reduction activity was higher in TMB3000 than in CBS8066. Petersson et al. (2006) performed a genome wide transcription analysis of known reductase and dehydrogenase genes of TMB3000 and CBS8066 cultured with 0.5 g/L HMF. TMB3000 showed higher expression levels for almost all of the reductase and dehydrogenase genes examined. Three of the eighteen genes studied were selected to further examine their role in HMF reduction. *ADH2*, *ADH6* and *SFA1* were the genes cloned from both TMB3000 and CBS8066 and overexpressed in CBS8066 using a vector construct (*107*).

The gene product of *ADH6* reduces HMF using NADPH as a cofactor. *ADH6* encodes an alcohol dehydrogenase that only uses NADPH as a cofactor; and it has high affinity for long chain aliphatic substrates (*108*). The *ADH6* gene product can also reduce furfural, cinnamaldehyde and veratraldehyde (*109*).The enhanced HMF reducing capacity of TMB3000 is not attributed to mutations in its coding region of *ADH6*, as similar HMF reducing capacities were observed with overexpression of *ADH6* isolated from either CBS8066 or TMB3000 (*107*). Sequence analysis confirms that the increased HMF reduction capacity of TMB3000 is not due to mutations in the coding region of its *ADH6*. Petersson et al. (2006) suggests that high expression of *ADH6* in TMB3000 could be due to mutations in regulator sequences.

ADH2 encodes an ethanol dehydrogenase and has high expression in TMB3000, but when overexpressed in CBS8066 there was no evidence of HMF reducing capacity (*107*). *SFA1* encodes an enzyme that converts aldehydes to ketones, by catalyzing amino acid catabolism (*110*). Overexpression of *SFA1* from CB8066 and TMB3000 in CB8066 resulted in improved HMF reduction activity.

Overexpression of *ADH6* in yeast when cultured in glucose media containing HMF resulted in increased glycerol yield under anaerobic and aerobic conditions, and decreased biomass yield under aerobic conditions (*107*). Decreased biomass yield is likely a result of a redirection of NADPH from biosynthetic pathways to HMF reduction, but increased glycerol yield is not well understood (*107*). Since HMF reduction is solely dependent on NADPH as a cofactor it is unexpected that an increase in NADPH production would result in increased glycerol production,

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

which is used to regenerate NAD+ for anabolic reactions. Other researchers reported that overexpression of *ADH6* in CBS8066 results in an increase in glycerol production, a decrease in biomass, and an increase in acetate production (*129*). HMF reduction by the NADPH-dependent enzyme encoded by *ADH6* places a demand for NADPH, which may be responsible for increased acetate, as production of acetate has a role in redox balancing and creation of acetate from acetaldehyde regenerates NADP+ (*129*, *130*).

In *S. cerevisiae* the pentose phosphate pathway (PPP) has been linked with furfural tolerance (*111*). The PPP is a central pathway in carbohydrate metabolism and is a primary source of NADPH (*131*). To identify genes involved in furfural tolerance, a *S. cerevisiae* gene disruption library was screened for mutants exhibiting decreased growth in the presence of furfural (*111*). It was observed that a number of mutants that were inefficient at reducing furfural were defective in the PPP. PPP disruption mutants of *S. cerevisiae* with deletions of PPP genes, *zwf1, gnd1, rpel* and *tkl1* displayed decreased growth in comparison to the control strain when grown with furfural. Overexpression of *zwf1*, encoding glucose-6 phosphate dehydrogenase, resulted in growth at concentrations of furfural up to 50 mM, a concentration toxic to *S. cerevisiae* strains not overexpressing *zwf1* (*111*). Increased levels of glucose-6 phosphate dehydrogenase, the first enzyme in the PPP, may commit its substrate, glucose-6 phosphate, to the PPP at the expense of other pathways (eg. glycolysis), resulting in high levels of NADPH (*111*). Although PPP's role in furfural tolerance has not yet been elucidated, it is likely due to increased NADPH, the cofactor of ADH6p and many other enzymes involved in stress response (*132*).

Recombinant xylose-fermenting *S. cerevisiae* strains have been created by insertion and overexpression of heterologous xylose isomerase, or xylose reductase and xylitol dehydrogenase (*6*). The fermentative ability of recombinant xylose-fermenting *S. cerevisiae* strains has been well characterized (*5*, *133–137*). One research group found enhanced tolerance to furfural and HMF by the xylose-fermenting *S. cerevisiae* TMP3400 strain, which expresses the *S. stipitis* xylose reductase (Ps-XR) (*112*). Strain TMB3400 is a mutant of a recombinant strain of *S. cerevisiae* USM21, which was created by chromosomal integration of *S. stipitis* genes encoding xylose reductase (XR) and xylitol dehydrogenase (XDH), and a *S. cerevisiae* gene encoding xylulokinase (XK) (*137*). TMB 3400 has higher tolerance to the inhibitors in spruce hydrolysate during anaerobic batch fermentation in comparison to control strain USM21, and has 3 times higher *in vitro* HMF and furfural reduction activity (*112*). Ps-XR is capable of both *in vitro* and *in vivo* HMF reduction. Creation of *S. cerevisiae* TMB3290, a new strain overexpressing Ps-XR, resulted in a 20% increase in the *in vivo* HMF reduction rate, and improved HMF tolerance (*112*).

Improved Phenol Tolerance

Exploiting *S. cerevisiae*'s innate ability to metabolize phenolic compounds to create strains that have increased tolerance has been a means to increase inhibitor tolerance (*138*). *S. cerevisiae*'s phenylacrylic acid decarboxylase is encoded by

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the *PAD1* gene, and its substrates include cinnamic, *p*-coumaric, and ferulic acids (*139*, *140*). Under aerobic conditions ferulic acid is converted to vinyl guaiacol; and under anaerobic conditions ferulic acid is converted to dihydroferulic acid (*138*)(*113*, *138*, *138*). Cinnamic acid is converted to styrene and dihydrocinnamic acid (*138*). The *in vivo* performance of genetically engineered *S. cerevisiae* strains overexpressing *PAD1* in the presence of ferulic and cinnamic acid containing media, and in spruce hydrolysate was examined (*138*). Overexpression of *PAD1* resulted in enhanced growth rate and ethanol productivity in the presence of cinnamic and ferulic acids, as well as in spruce hydrolysate. *S. cerevisiae* overexpressing *PAD1* converted ferulic acid 1.5 times, and cinnamic acid 4 times faster than the control yeast. *PAD1* overexpression led to yeast consuming glucose and mannose at rates 22-25% and 40-45%, respectively, faster than yeast not overexpressing *PAD1*. Additionally, ethanol production rate was 24-29% faster in yeast overexpressing *PAD1*.

Heterologous expression of the *Trametes versicolor* laccase gene in a selection of yeast species has been explored as a means of increasing yeast tolerance to phenolic compounds (*86*, *113*, *114*). Previous research (described above) has focused on in vitro application of the laccase enzyme to lignocellulosic hydrolysates as a detoxification step to remove phenolic compounds (*86*, *114*). However, it is advantageous to bypass costly detoxification steps. Expression of laccase in yeast species improves their tolerance to phenolic compounds and reduces reliance on costly detoxification steps.

Early research focused on determining what factors were essential for secretion of active laccase (*86*, *114*). The laccase gene, *lcc1* was cloned into a vector and expressed in the methylotrophic yeast *Pichia pastoris* (*86*). Transformed *P. pastoris* did secrete active laccase. A pH range of 4 to 6 of the culture media (*85*, *86*), and lower temperature (19 °C) (*114*) were found to be important to obtain active enzymes. Although P. *pastoris* has high levels of protein secretion, and is commonly used for heterologous protein expression, it does not efficiently produce ethanol from lignocellulosic hydrolysates due to the strain's high oxygen requirement (*141*). Expression of laccase in ethanologenic yeast, such as *S. cerevisiae* is a more realistic long-term goal. *lcc2*, the gene encoding an isoenzyme of laccase A, was expressed in *S. cerevisiae* (*114*). Similar to expression of *lcc1* in *P. pastoris*, low culture temperatures were vital for obtaining active laccase enzymes. After 3 days of incubation, transformed *S. cerevisiae* grown at 19°C had 16 times higher laccase activity than that grown at 28°C.

Laccase, when expressed under control of the *PCK1* promoter in *S. cerevisiae* exhibited a 2-fold increase in activity when a homologous *t*-SNARE protein, Sso2p was simultaneously overexpressed (*138*). Sso2p is a membrane protein associated with protein secretion in *S. cerevisiae*, and is implicated as a rate-limiting step in protein secretion (*142*). Laccase transformants overexpressing Sso2p exhibited increased laccase activity irrespective of culture conditions. Similar to expression in *P. pastoris*, low pH of culture medium reduced laccase activity. Laccase activity was enhanced with large culture volumes and low aeration, which is advantageous for use in the oxygen limited conditions of ethanol production from lignocellulosic hydrolysates. *S. cerevisiae* overexpressing Sso2p and expressing laccase were able

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to ferment a dilute-acid spruce hydrolysate with a faster aldehyde conversion rate, and had greater ethanol productivity than the control yeast strain. The ethanol yield for the transformed yeast when fermenting the hydrolysate was 0.44 g/g; in comparison the control yeast's ethanol yield was 0.02 g/g. The spruce hydrolysate contained (per L) 20.5 g glucose, 14.9 g mannose, 7.0 g xylose, 2.9 g galactose, 1.4 g arabinose, 2.8 g acetic acid, 0.7 g formic acid, 1.1 g levulinic acid, 1.4 g furfural, and 2.3 g HMF, and 2.9 g phenolic compounds. The transformed yeast was also able to grow in a Sc-URA medium containing coniferyl aldehyde at a concentration (1.25 mmol/L). The control yeast was not able to grow on this medium. There was a 20 hour lag phase during which the coniferyl aldehyde concentration decreased to 0.6 mmol/L. After the concentration of coniferyl aldehyde reached below 0.6 mmol/L cell growth commenced and ethanol production rate increased, resulting in a final ethanol yield of 0.44 g/g. No ethanol was produced by the control yeast.

Strain engineering represents an effective tool to improve tolerance of yeasts to inhibitors with the end result of improved ethanol production from lignocellulosic substrates. Recombinant yeast strains created to date have increased tolerance to specific inhibitors. As more inhibitor-metabolizing enzymes are indentified, and a better understanding of the stress response of yeast is gained, even more tolerant recombinant yeasts can be engineered for optimal fermentation of lignocellulosic hydrolysates.

Evolutionary Engineering of Yeasts for Improved Inhibitor Tolerance

Evolutionary engineering is a method mimicking natural selection through directed evolution to obtain a desired phenotype. The goal of directed evolution is improvement of cellular properties through iterative genetic diversification (mutagen induced or not) and functional selection (*143*). Unlike molecular techniques used to create recombinant yeasts, evolutionary engineering does not require prior knowledge of the genes involved in a desired phenotype, or the modifications needed to generate the desired phenotype. Evolutionary engineering has been used to increase yeast tolerance to inhibitors in lignocellulosic hydrolysates (*115*, *116*). Although yeast tolerance to inhibitors can be increased through continuous adaptation by growing the yeast in the hydrolysate (*144*), it is time-consuming. Random mutagenesis can quickly generate mutant yeast strains that show improved inhibitor tolerance (*116*). UV mutagenesis has been used to generate mutants of *S. stipitis* NRRL Y-7124 that exhibited improved tolerance to inhibitors in hardwood spent sulfite liquor (HW SSL) (*116*). A UV dose causing 50% cell survival and then selection upon gradient plates of HW SSL and diluted liquid HW SSL were used to generate and select mutants tolerant of the mixed inhibitors in HW SSL. The *S. stipitis* mutants were able to grow in 75% (v/v) HW SSL broth, whereas the wild type (WT) was only able to grow in 65% (v/v) HW SSL. Two of the six, 3rd generation mutants, PS301 and PS302, survived in 80% (v/v) HW SSL, but no growth was observed. Compared to the WT, the growth rate was slightly increased for the mutants, but the mutants had lower growth yields on xylose and glucose. The mutants displayed improved fermentation efficiency on 4% (w/v) xylose, and the same fermentation efficiency on 4% (w/v) glucose compared to the WT. The mutants had a higher ethanol yield than the WT when a mixture of 4% (w/v) each of glucose and xylose was fermented. After four days of fermentation, the mutants utilized 100% of the xylose in the medium, whereas the WT only utilized 26.2% of the xylose. The reason for improved xylose utilization of the mutants is not known (*116*). Glucose repression of xylose utilization, where glucose is utilized before xylose, is a common problem with mixed sugar fermentations (*12*). The mutants started to utilize xylose when 1% glucose remained in the fermentation medium (*116*). Additionally, in 60% (v/v) HW SSL, the mutants were able to utilize and ferment glucose, mannose, galactose and xylose, while the WT was unable to ferment any of the sugars.

Subsequent research focused on the use of genome shuffling of the *S. stipitis* mutants to further improve HW SSL tolerance (*115*).Whole genome shuffling is a technique using iterative cycles of multi-parental crossing with recombination of entire genomes to generate single cells with useful alleles expressing an improved phenotype (*145*). Genome shuffling is an appropriate technique to use to improve complex mutli-genic phenotypic traits not easily modified by traditional molecular techniques. Genome shuffling may prove to be a very useful tool in the creation of yeast strains that are highly tolerant to a variety of inhibitors as individual mutants expressing alleles conveying resistance to a particular inhibitor can be combined in one cell to create a multi-inhibitor tolerant strain. The use of genome shuffling should ideally result in an accumulation of beneficial mutations and elimination of deleterious mutations. Bajwa et al. (2010) created genome shuffled mutants of *S. stipitis* by starting with a pool of UV-induced mutants displaying improved HW SSL tolerance. The mutants were recombined using sexual mating followed by sporulation to regenerate the haploid cells. After each round of genome shuffling growth on HW SSL gradient plates was used to select improved mutant strains to serve as the starting pool for the next round of genome shuffling. A total of four rounds of genome shuffling were completed. Improved growth in liquid HW SSL was observed for 4 of the mutants. GS301 and GS302, from the third round of genome shuffling, and GS401 and GS402 from the fourth round, were able to grow in 80% (v/v) HW SSL (*115*). GS301 and GS301 displayed the best HW SSL tolerance as they were able to grow in 85% (v/v) HW SSL, with GS301 showing viability in 90% (v/v), but no growth was observed. Fermentations of a mixture of 4% (w/v) each of glucose and xylose using GS301 and GS301 were carried out. GS301 and GS302 utilized glucose and xylose more efficiently and produced more ethanol than the WT. Both strains utilized all the glucose within 20 hours, while the WT took 36 hours to consume all the glucose. Due to glucose repression, xylose was poorly utilized by the WT, taking 150 hours to consume only 1% (w/v). The genome shuffled strains consumed all the xylose in about 108 hours. The maximum ethanol concentration accumulated by GS301 and GS302 ranged from 2.8 to 2.9% (w/v), while for the WT it was only 1.8% (w/v). The genome shuffled strains produced low levels of ethanol, 0.15 to 0.18% (w/v), from the sugars in undiluted HW SSL, but the WT was unable to utilize or ferment any of the sugars. This research demonstrated genome shuffling of *S. stipitis* can be used for the improvement of HW SSL tolerance.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

Lignocellulosic hydrolysates contain a large assortment of inhibitors, which have synergistic effects on yeast cells. Overcoming the synergistic effects of multiple inhibitors will likely require the creation of a genome shuffled *S. stipitis* strain that combines mutations that confer tolerance to key inhibitors in hydrolysates (*115*). Since HW SSL has many of the same inhibitors found in many of the lignocellulosic hydrolysates, it is hypothesized that the genome shuffled strains from Bajwa et al. (2010) would be tolerant of inhibitors in other lignocellulosic hydrolysates. This remains to be tested.

Summary

The recalcitrance of lignocellulosic materials requires pretreatment processes to make the sugar monomers available for fermentation. The pretreatment processes generate a range of inhibitory compounds that are toxic to the microorganisms used for fermentation. The inhibitors in lignocellulosic hydrolysates may act synergistically to hinder the growth and viability of the fermenting microorganisms and reduce rates of ethanol production and ethanol yields.

Approaches to overcome this problem involve removal of inhibitors or converting them to less toxic forms through a detoxification step prior to fermentation. Physical detoxification methods have focused on removing the inhibitors through the use of ion exchange resins, activated carbon, vacuum evaporation, liquid extractions and lignin treatment. Alkali treatment is a commonly used chemical detoxification method, which is one of the least expensive. Biological detoxification methods have focused on bioabatement of the inhibitors in lignocellulosic hydrolysates by using other microorganisms, or by treating the hydrolysates with laccase. A detoxification step prior to fermentation represents added cost and time to the bioconversion process. Ideally, economical production of lignocellulosic ethanol will not require a detoxification step. Efforts should be directed at alternative methods of pretreatment which reduce the amount of inhibitors being generated.

Fed batch and continuous fermentation strategies have been used to produce ethanol from lignocellulosic hydrolysates without a detoxification step. Through the use of yeast strains more tolerant to inhibitors, *in situ* detoxification of hydrolysates has been possible using fed batch or continuous modes of fermentation. The greatest improvement and most practical option for the production of industrial scale lignocellulosic ethanol relies on obtaining yeast strains that tolerate high concentrations of a variety of inhibitors. Traditional adaptation methods of subculturing yeast cells with increasing concentrations of hydrolysates have been used to generate yeasts able to ferment undetoxified hydrolysates. However, traditional adaptation methods are time-consuming. Recombinant techniques have been used to create yeast strains that are more tolerant to selected inhibitors through the overexpression or elimination of key genes conferring inhibitor tolerance. The complex nature of the genes involved in yeast stress response and tolerance to all the different inhibitors makes recombinant techniques not suitable. Recombinant techniques require isolating

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the tolerance-conferring genes and knowledge of what changes are needed in the genes in order to create a more tolerant yeast strain. In the absence of knowledge of the mechanism of inhibitor tolerance, research efforts have focused on using evolutionary engineering and genome shuffling to obtain yeast strains with increased inhibitor and ethanol tolerance, while decreasing hexose represson. Genome shuffling is a useful technique to improve complicated multi-genic phenotypic traits not easily amenable to modification by traditional molecular techniques, as no prior knowledge of which set of genes to modify the increased tolerance is required.

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References

1. Environment Canada. http://www.ec.gc.ca/envirozine/default.asp?lang=En&n=AB656AC7-1) (accessed 07/31/10), Taming Transport: New Regulations for Greenhouse Gas Emissions.
2. Environment Canada. http://www.ec.gc.ca/ceparegistry/documents/participation/renewable_fuels/default.cfm (accessed 07/31/10), Renewable Fuels.
3. Natural Resources Canada. http://oee.nrcan.gc.ca/transportation/fuels/ethanol/benefits.cfm?attr=8 (accessed 07/31/10), Benefits of ethanol.
4. Champagne, P. *Environ. Prog.* **2008**, *27*, 51–57.
5. Hahn-Hagerdal, B.; Galbe, M.; Gorwa-Grauslund, M. F.; Liden, G.; Zacchi, G. *Trends Biotechnol.* **2006**, *24*, 549–556.
6. Chu, B. C. H.; Lee, H. *Biotechnol. Adv.* **2007**, *25*, 425–441.
7. Saha, B. C. *J. Ind. Microbiol. Biotechnol.* **2003**, *30*, 279–291.
8. Webb, S. R.; Lee, H. *Biotechnol. Adv.* **1990**, *8*, 685–697.
9. Gray, K. A.; Zhao, L. S.; Emptage, M. *Curr. Opin. Chem. Biol.* **2006**, *10*, 141–146.
10. Bicho, P. A.; Runnals, P. L.; Cunningham, J. D.; Lee, H. *Appl. Environ. Microbiol.* **1988**, *54*, 50–54.
11. Batt, C. A.; Carvallo, S.; Easson, D. D.; Akedo, M.; Sinskey, A. J. *Biotechnol. Bioeng.* **1986**, *28*, 549–553.
12. Lee, H. *FEMS Microbiol. Lett.* **1992**, *92*, 1–4.
13. Zhao, L.; Zhang, X.; Tan, T. *Biomass Bioenergy* **2008**, *32*, 1156–1161.
14. Lohmeier-Vogel, E. M.; Sopher, C. R.; Lee, H. *J. Ind. Microbiol. Biotechnol.* **1998**, *20*, 75–81.
15. Barbosa, M. D. S.; Lee, H.; Collins-Thompson, D. L. *Appl. Environ. Microbiol.* **1990**, *56*, 545–550.
16. Lindén, T.; Hahn-Hägerdal, B. *Enzyme Microb. Technol.* **1989**, *11*, 583–589.
17. Klinke, H. B.; Thomsen, A. B.; Ahring, B. K. *Appl. Microbiol. Biotechnol.* **2004**, *66*, 10–26.
18. Olsson, L.; Hahn-Hagerdal, B. *Enzyme Microb. Technol.* **1996**, *18*, 312–331.
19. Saha, B. C. *J. Ind. Microbiol. Biotechnol.* **2003**, *30*, 279–291.
20. du Preez, J. C. *Enzyme Microb. Technol.* **1994**, *16*, 944–956.
21. Lee, H.; To, R. J. B.; Latta, R. K.; Biely, P.; Schneider, H. *Appl. Environ. Microbiol.* **1987**, *53*, 2831–2834.

22. Lee, Y. Y.; McCaskey, T. A. *Tappi J.* **1983**, *66*, 102–107.
23. Watson, N. E.; Prior, B. A.; Lategan, P. M.; Lussi, M. *Enzyme Microb. Technol.* **1984**, *6*, 451–456.
24. Lu, Y. L.; Warner, R.; Sedlak, M.; Ho, N.; Mosier, N. S. *Biotechnol. Prog.* **2009**, *25*, 349–356.
25. Taherzadeh, M. J.; Niklasson, C.; Lidén, G. *Chem. Eng. Sci.* **1997**, *52*, 2653–2659.
26. Palmqvist, E.; Hahn-Hagerdal, B. *Bioresour. Technol.* **2000**, *74*, 25–33.
27. McMillan, J. D. Conversion of Hemicellulose of Hydrolysates to Ethanol. In *Enzymatic Conversion of Biomass for Fuels Production*; Himmel, M. E., Baker, J. O., Overend, R. P., Eds.; ACS Symposium Series 566; American Chemical Society: Washington, DC, 1994; pp 411−437.
28. Clark, T. A.; Mackie, K. L. *J. Chem. Technol. Biotechnol., Biotechnol.* **1984**, *34*, 101–110.
29. Pampulha, M. E.; Loureirodias, M. C. *Appl. Microbiol. Biotechnol.* **1989**, *31*, 547–550.
30. Larsson, S.; Reimann, A.; Nilvebrant, N. O.; Jönsson, L. J. *Appl. Biochem. Biotechnol.* **1999**, *77-79*, 91–103.
31. Casey, E.; Sedlak, M.; Ho, N. W. Y.; Mosier, N. S. *FEMS Yeast Res.* **2010**, *10*, 385–393.
32. Verduyn, C.; Postma, E.; Scheffers, W. A.; Vandijken, J. P. *J. Gen. Microbiol.* **1990**, *136*, 405–412.
33. Palmqvist, E.; Hahn-Hagerdal, B. *Bioresour. Technol.* **2000**, *74*, 17–24.
34. Helle, S.; Cameron, D.; Lam, J.; White, B.; Duff, S. *Enzyme Microb. Technol.* **2003**, *33*, 786–792.
35. Diaz, M.; Ruiz, E.; Romero, I.; Cara, C.; Moya, M.; Castro, E. *World J. Microb. Biot.* **2009**, *25*, 891–899.
36. Cho, D. H.; Shin, S.; Bae, Y.; Park, C.; Kim, Y. H. *Bioresour. Technol.* **2010**, *101*, 4947–4951.
37. Wilson, J. J.; Deschatelets, L.; Nishikawa, N. K. *Appl. Microbiol. Biotechnol.* **1989**, *31*, 592–596.
38. Almeida, J. R. M.; Modig, T.; Petersson, A.; Hahn-Hagerdal, B.; Liden, G.; Gorwa-Grauslund, M. F. *J. Chem. Technol. Biotechnol.* **2007**, *82*, 340–349.
39. Chung, I. S.; Lee, Y. Y. *Biotechnol. Bioeng.* **1985**, *27*, 308–315.
40. Taherzadeh, M. J.; Gustafsson, L.; Niklasson, C.; Liden, G. *J. Biosci. Bioeng.* **1999**, *87*, 169–174.
41. Banerjee, N.; Bhatnagar, R.; Viswanathan, L. *Eur. J. Appl. Microbiol.* **1981**, *11*, 226–228.
42. Palmqvist, E.; Almeida, J. S.; Hahn-Hagerdal, B. *Biotechnol. Bioeng.* **1999**, *62*, 447–454.
43. Navarro, A. R. *Curr. Microbiol.* **1994**, *29*, 87–90.
44. Sanchez, B.; Bautista, J. *Enzyme Microb. Technol.* **1988**, *10*, 315–318.
45. Banerjee, N.; Bhatnagar, R.; Viswanathan, L. *Enzyme Microb. Technol.* **1981**, *3*, 24–28.
46. Horvath, I. S.; Sjöde, A.; Nilvebrant, N. O.; Zagorodni, A.; Jönsson, L. J. *Appl. Biochem. Biotechnol.* **2004**, *113-116*, 525–538.

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47. Taherzadeh, M. J.; Eklund, R.; Gustafsson, L.; Niklasson, C.; Liden, G. *Ind. Eng. Chem. Res.* **1997**, *36*, 4659–4665.
48. Öhgren, K.; Bengtsson, O.; Gorwa-Grauslund, M. F.; Galbe, M.; Hahn-Hägerdal, B.; Zacchi, G. *J. Biotechnol.* **2006**, *126*, 488–498.
49. Nigam, J. N. *J. Ind. Microbiol. Biotechnol.* **2001**, *26*, 145–150.
50. Palmqvist, E.; Grage, H.; Meinander, N. Q.; Hahn-Hagerdal, B. *Biotechnol. Bioeng.* **1999**, *63*, 46–55.
51. Zaldivar, J.; Martinez, A.; Ingram, L. O. *Biotechnol. Bioeng* **1999**, *65*, 24–33.
52. Taherzadeh, M. J.; Gustafsson, L.; Niklasson, C.; Liden, G. *Appl. Microbiol. Biotechnol.* **2000**, *53*, 701–708.
53. Sikkema, J.; Debont, J. A. M.; Poolman, B. *Microbiol. Rev.* **1995**, *59*, 201–222.
54. Delgenes, J. P.; Moletta, R.; Navarro, J. M. *Enzyme Microb. Technol.* **1996**, *19*, 220–225.
55. Kelly, C.; Jones, O.; Barnhart, C.; Lajoie, C. *Appl. Biochem. Biotechnol.* **2008**, *148*, 97–108.
56. Mills, T. Y.; Sandoval, N. R.; Gill, R. T. *Biotechnol. Biofuels* **2009**, *2*, 11–21.
57. Cantarella, M.; Cantarella, L.; Gallifuoco, A.; Spera, A.; Alfani, F. *Biotechnol. Prog.* **2004**, *20*, 200–206.
58. Tran, A. V.; Chambers, R. P. *Biotechnol. Lett.* **1985**, *7*, 841–845.
59. Persson, P.; Andersson, J.; Gorton, L.; Larsson, S.; Nilvebrant, N.; Jönsson, L. J. *J. Agric. Food Chem.* **2002**, *50*, 5318–5325.
60. Sivers, M. v.; Zacchi, G.; Olsson, L.; Hahn-Hägerdal, B. *Biotechnol. Prog.* **1994**, *10*, 555–560.
61. Palmqvist, E.; Hahn-Hägerdal, B.; Galbe, M.; Zacchi, G. *Enzyme Microb. Technol.* **1996**, *19*, 470–476.
62. Cantarella, M.; Cantarella, L.; Gallifuoco, A.; Spera, A.; Alfani, F. *Process Biochem.* **2004**, *39*, 1533–1542.
63. Cruz, J. M.; Domínguez, J. M.; Domínguez, H.; Parajó, J. C. *Food Chem.* **1999**, *67*, 147–153.
64. Chandel, A. K.; Kapoor, R. K.; Singh, A.; Kuhad, R. C. *Bioresour. Technol.* **2007**, *98*, 1947–1950.
65. Nilvebrant, N. O.; Reimann, A.; Jönsson, L. J. *Appl. Biochem. Biotechnol.* **2001**, *91-93*, 35–49.
66. Berson, R. E.; Young, J. S.; Kamer, S. N.; Hanley, T. R. *Appl. Biochem. Biotechnol.* **2005**, *121-124*, 923–934.
67. Priddy, S. A.; Hanley, T. R. *Appl. Biochem. Biotechnol.* **2003**, *105-108*, 353–364.
68. Bjorklund, L.; Larsson, S.; Jönsson, L. J.; Reimann, A.; Nilvebrant, N. O. *Appl. Biochem. Biotechnol.* **2002**, *98-100*, 563–575.
69. Alriksson, B.; Horvath, I. S.; Sjöde, A.; Nilvebrant, N. O.; Jönsson, L. J. *Appl. Biochem. Biotechnol.* **2005**, *121-124*, 911–922.
70. Grohmann, K.; Torget, R.; Himmel, M. *Biotechnol. Bioeng* **1985**, *15*, 59–80.
71. Horvath, I. S.; Sjöde, A.; Alriksson, B.; Jönsson, L. J.; Nilvebrant, N. O. *Appl. Biochem. Biotechnol.* **2005**, *121-124*, 1031–1044.
72. Leonard, R. H.; Hajny, G. J. *Ind. Eng. Chem.* **1945**, *37*, 390–395.

Downloaded by PENNSYLVANIA STATE UNIV on June 17, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch007

73. Martinez, A.; Rodriguez, M. E.; York, S. W.; Preston, J. F.; Ingram, L. O. *Biotechnol. Bioeng.* **2000**, *69*, 526–536.
74. Martinez, A.; Rodriguez, M. E.; Wells, M. L.; York, S. W.; Preston, J. F.; Ingram, L. O. *Biotechnol. Prog.* **2001**, *17*, 287–293.
75. Nilvebrant, N. O.; Persson, P.; Reimann, A.; De Sousa, F.; Gorton, L.; Jönsson, L. J. *Appl. Biochem. Biotechnol.* **2003**, *105-108*, 615–628.
76. Perego, P.; Converti, A.; Palazzi, E.; Del Borghi, M.; Ferraiolo, G. *J. Ind. Microbiol.* **1990**, *6*, 157–164.
77. Persson, P.; Larsson, S.; Jönsson, L. J.; Nilvebrant, N. O.; Sivik, B.; Munteanu, F.; Thörneby, L.; Gorton, L. *Biotechnol. Bioeng.* **2002**, *79*, 694–700.
78. Sjolander, N. O.; Langlykke, A. F. *Ind. Eng. Chem.* **1938**, *30*, 1251–1255.
79. Taherzadeh, M. J.; Niklasson, C.; Lidén, G. *Chem. Eng. Sci.* **1997**, *52*, 2653–2659.
80. Lopez, M. J.; Nichols, N. N.; Dien, B. S.; Moreno, J. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 125–131.
81. Nichols, N. N.; Dien, B. S.; Guisado, G. M.; López, M. J. *Appl. Biochem. Biotechnol.* **2005**, *121*, 379–390.
82. Nichols, N. N.; Sharma, L. N.; Mowery, R. A.; Chambliss, C. K.; van Walsum, G. P.; Dien, B. S.; Iten, L. B. *Enzyme Microb. Technol.* **2008**, *42*, 624–630.
83. Okuda, N.; Soneura, M.; Ninomiya, K.; Katakura, Y.; Shioya, S. *J. Biosci. Bioeng.* **2008**, *106*, 128–133.
84. Schneider, H. *Enzyme Microb. Technol.* **1996**, *19*, 94–98.
85. Jönsson, L. J.; Sjöström, K.; Häggström, I.; Nyman, P. O. *Biochim. Biophys. Acta* **1995**, *1251*, 210–215.
86. Jönsson, L. J.; Saloheimo, M.; Penttilä, M. *Curr. Genet.* **1997**, *32*, 425–430.
87. Jönsson, L. J.; Palmqvist, E.; Nilvebrant, N. O.; Hahn-Hägerdal, B. *Appl. Microbiol. Biotechnol.* **1998**, *49*, 691–697.
88. Jurado, M.; Prieto, A.; Martínez-Alcalá, Á.; Martínez, Á. T.; Martínez, M. J. *Bioresour. Technol.* **2009**, *100*, 6378–6384.
89. Rudolf, A.; Galbe, M.; Liden, G. *Appl. Biochem. Biotechnol.* **2004**, *114*, 601–617.
90. Taherzadeh, M. J.; Niklasson, C.; Lidén, G. *Biotechnol. Bioeng.* **2000**, *69*, 330–338.
91. Taherzadeh, M. J.; Gustafsson, L.; Niklasson, C.; Liden, G. *Appl. Microbiol. Biotechnol.* **2000**, *53*, 701–708.
92. Taherzadeh, M. J.; Gustafsson, L.; Niklasson, C.; Liden, G. *J. Biosci. Bioeng.* **2000**, *90*, 374–380.
93. Taherzadeh, M. J.; Niklasson, C.; Liden, G. *Bioresour. Technol.* **1999**, *69*, 59–66.
94. Brandberg, T.; Sanandaji, N.; Gustafsson, L.; Franzen, C. J. *Biotechnol. Prog.* **2005**, *21*, 1093–1101.
95. De Bari, I.; Cuna, D.; Nanna, F.; Braccio, G. *Appl. Biochem. Biotechnol.* **2004**, *114*, 539–557.
96. Horvath, I. S.; Taherzadeh, M. J.; Niklasson, C.; Lidén, G. *Biotechnol. Bioeng.* **2001**, *75*, 540–549.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch007

97. Lee, W. G.; Lee, J. S.; Park, B. G.; Chang, H. N. *Korean J. Chem. Eng.* **1996**, *13*, 453–456.
98. Palmqvist, E.; Galbe, M.; Hahn-Hägerdal, B. *Appl. Biochem. Biotechnol.* **1998**, *50*, 545–551.
99. Purwadi, R.; Taherzadeh, M. J. *Bioresour. Technol.* **2008**, *99*, 2226–2233.
100. Purwadi, R.; Brandberg, T.; Taherzadeh, M. J. *Int. J. Mol. Sci.* **2007**, *8*, 920–932.
101. Talebnia, F.; Taherzadeh, M. J. *J. Biotechnol.* **2006**, *125*, 377–384.
102. Alkasrawi, M.; Rudolf, A.; Liden, G.; Zacchi, G. *Enzyme Microb. Technol.* **2006**, *38*, 279–286.
103. Amartey, S.; Jeffries, T. W. *J. Microbiol. Biotechnol.* **1996**, *12*, 281–283.
104. Keller, F. A.; Bates, D.; Ruiz, R.; Nguyen, Q. *Appl. Biochem. Biotechnol.* **1998**, *70-72*, 137–147.
105. Martin, C.; Marcet, M.; Almazan, O.; Jonsson, L. J. *Bioresour. Technol.* **2007**, *98*, 1767–1773.
106. Parekh, S. R.; Yu, S.; Wayman, M. *Appl. Microbiol. Biotechnol.* **1986**, *25*, 300–304.
107. Petersson, A.; Almeida, J. R. M.; Modig, T.; Karhumaa, K.; Hahn-Hagerdal, B.; Gorwa-Grauslund, M. F.; Lidén, G. *Yeast* **2006**, *23*, 455–464.
108. Larroy, C.; Fernandez, M. R.; Gonzalez, E.; Pares, X.; Biosca, J. A. *Chem. Biol. Interact.* **2003**, *143*, 229–238.
109. Larroy, C.; Fernandez, M. R.; Gonzalez, E.; Pares, X.; Biosca, J. A. *Biochem. J.* **2002**, *361*, 163–172.
110. Dickinson, J. R.; Eshantha, L.; Salgado, J.; Hewlins, M. J. E. *J. Biol. Chem.* **2003**, *278*, 8028–8034.
111. Gorsich, S. W.; Dien, B. S.; Nichols, N. N.; Slininger, P. J.; Liu, Z. L.; Skory, C. D. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 339–349.
112. Almeida, J.; Modig, T.; Röder, A.; Lidén, G.; Gorwa-Grauslund, M. *Biotechnol. Biofuel* **2008**, *1*, 12–20.
113. Larsson, S.; Cassland, P.; Jönsson, L. J. *Appl. Environ. Microbiol.* **2001**, *67*, 1163–1170.
114. Cassland, P.; Jönsson, L. J. *Appl. Microbiol. Biotechnol.* **1999**, *52*, 393–400.
115. Bajwa, P. K.; Pinel, D.; Martin, V. J. J.; Trevors, J. T.; Lee, H. *J. Microbiol. Methods* **2010**, *81*, 179–186.
116. Bajwa, P. K.; Shireen, T.; D'Aoust, F.; Pinel, D.; Martin, V. J. J.; Trevors, J. T.; Lee, H. *Biotechnol. Bioeng.* **2009**, *104*, 892–900.
117. López, M. J.; Nichols, N. N.; Dien, B. S.; Moreno, J.; Bothast, R. J. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 125–131.
118. Lobo, Z.; Maitra, P. K. *Biotechnol. Bioeng.* **1977**, *86*, 727–744.
119. Ma, H.; Botstein, D. *Mol. Cell Biol.* **1986**, *6*, 4046–4052.
120. Ballesteros, I.; Negro, M. J.; Manzaneres, P.; Oliva, J. M.; Sáez, F.; Ballesteros, M. *Appl. Biochem. Biotechnol.* **2006**, *129-132*, 496–508.
121. Kabel, M. A.; Bos, G.; Zeevalking, J.; Voragen, A. G. J.; Schols, H. A. *Bioresour. Technol.* **2007**, *98*, 2034–2042.
122. Qian, M.; Tian, S.; Li, X.; Zhang, J.; Pan, Y.; Yang, X. *Appl. Biochem. Biotechnol.* **2006**, *134*, 273–283.
123. Nilsson, A.; Taherzadeh, M. J.; Lidén, G. *J. Biotechnol.* **2001**, *89*, 41–53.

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124. Nilsson, A.; Taherzadeh, M. J.; Liden, G. *Bioprocess. Biosyst. Eng.* **2002**, *25*, 183–191.
125. Kishimoto, M.; Nitta, Y.; Kamoshita, Y.; Suzuki, T.; Suga, K. I. *J. Ferment. Bioeng.* **1997**, *84*, 449–454.
126. Brandberg, T.; Franzén, C. J.; Gustafsson, L. *J. Biosci. Bioeng.* **2004**, *98*, 122–125.
127. Modig, T.; Almeida, J. R. M.; Gorwa-Grauslund, M. F.; Lidén, G. *Biotechnol. Bioeng.* **2008**, *100*, 423–429.
128. Nilsson, A.; Gorwa-Grauslund, M. F.; Hahn-Hägerdal, B.; Lidén, G. *Appl. Environ. Microbiol.* **2005**, *71*, 7866–7871.
129. Almeida, J.; Röder, A.; Modig, T.; Laadan, B.; Lidén, G.; Gorwa-Grauslund, M. F. *Appl. Microbiol. Biotechnol.* **2008**, *78*, 939–945.
130. van Dijken, J. P.; Scheffers, W. A. *FEMS Microbiol. Lett.* **1986**, *32*, 199–224.
131. Flores, C. L.; Rodriguez, C.; Petit, T.; Gancedo, C. *FEMS Microbiol. Rev.* **2000**, *24*, 507–529.
132. Carmel-Harel, O.; Storz, G. *Annu. Rev. Microbiol.* **2000**, *54*, 439–461.
133. Amore, R.; Kötter, P.; Küster, C.; Ciriacy, M.; Hollenberg, C. P. *Gene* **1991**, *109*, 89–97.
134. Hahn-Hägerdal, B.; Karhumaa, K.; Fonseca, C.; Spencer-Martins, I.; Gorwa-Grauslund, M. F. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 937–953.
135. Sedlak, M.; Ho, N. W. Y. *Appl. Biochem. Biotechnol.* **2004**, *113-116*, 403–416.
136. Takuma, S.; Nakashima, N.; Tantirungkij, M.; Kinoshita, S.; Okada, H.; Seki, T.; Yoshida, T. *Appl. Biochem. Biotechnol.* **1991**, *28-29*, 327–340.
137. Wahlbom, C. F.; van Zyl, W. H.; Jönsson, L. J.; Hahn-Hägerdal, B.; Otero, R. R. C. *FEMS Yeast Res.* **2003**, *3*, 319–326.
138. Larsson, S.; Nilvebrant, N. O.; Jönsson, L. J. *Appl. Microbiol. Biotechnol.* **2001**, *57*, 167–174.
139. Goodey, A. R.; Tubb, R. S. *J. Gen. Microbiol.* **1982**, *128*, 2615–2620.
140. Clausen, M.; Lamb, C. J.; Megnet, R.; Doerner, P. W. *Gene* **1994**, *142*, 107–112.
141. Romanos, M. A.; Scorer, C. A.; Clare, J. J. *Yeast* **1992**, *8*, 423–488.
142. Ruohonen, L.; Toikkanen, J.; Tieaho, V.; O utola, M.; Soderlund, H.; Keranen, S. *Yeast* **1997**, *13*, 337–351.
143. Koffas, M.; Cardayre, S. d. *Metab. Eng.* **2005**, *7*, 1–3.
144. Nigam, J. N. *J. Ind. Microbiol. Biotech.* **2001**, *26*, 145–150.
145. Zhang, Y. X.; Perry, K.; Vinci, V. A.; Powell, K.; Stemmer, W. P. C.; del Cardayré, S. B. *Nature* **2002**, *415*, 644–646.

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Chapter 8

Hydrogen Production from Carbohydrates: A Mini-Review

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The hydrogen economy promises a clean energy future featuring higher energy utilization efficiency and fewer pollutants compared to liquid fuel/internal combustion engines. Hydrogen production from the enriched low-cost biomass carbohydrates would achieve nearly zero carbon emissions in a whole life cycle. In this book chapter, we present latest advances of hydrogen generation from biomass carbohydrates by chemical catalysis (e.g., gasification, pyrolysis, gasification in supercritical water, and aqueous phase reforming), biocatalysis (e.g., anaerobic fermentation, electrohydrogenesis, photo-fermentation, and cell-free synthetic pathway biotransformation – SyPaB), and their combinations. Since hydrogen yield or energy efficiency is the most critical economic factor for hydrogen generation, SyPaB that can produce 12 H_2 per glucose equivalent seems to be an ultimate winner. When more stable enzyme building blocks with total turn-over number (TTN) values of more than 3×10^7 mol of product per mol of enzyme and engineered redox enzymes that can use low-cost stable biomimetic cofactor are available, cell-free SyPaB would produce hydrogen at the overall costs of less than \$2 kg of hydrogen.

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

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Introduction

"What will replace cheap oil -- and when?" was listed as one of top 25 questions by Science Magazine in the year 2005 (*1*). Crude oil is a modern industrial blood, which is converted to affordable liquid fuels (e.g., gasoline, diesel, jet fuel) for the transportation sector, and other derivatives (e.g., plastics, heat oil, and lubricants). Since more than 70% of crude oil is consumed in the transportation sectors, it is vital to find out sustainable transportation fuel alternatives to replace liquid fuels that are usually used in internal combustion engines (ICE).

Combustion of fossil fuels results in net emissions of greenhouse gases, air pollution, as well as concerns of energy security, wealth transfer, trade deficits, and health problems (*2*, *3*). Therefore, reconstruction of a sustainable energy system to remedy the depletion of oil and its negative environmental impacts have become two of the most critical issues to current scientists and engineers (*4*, *5*).

Hydrogen is the most common element in the universe, but there is nearly no dihydrogen source on the Earth. Hydrogen atoms are present in water (most) and other H-containing compounds (e.g., carbohydrate and hydrocarbons). Dihydrogen gas is considered as a promising energy carrier to replace fossil fuel-based liquid fuels, offering advantages through hydrogen fuel cell systems, such as nearly no air pollution, high energy conversion efficiency, diverse primary energy sources, plus recyclable water as a hydrogen source (*6*, *7*).

Low-cost renewable carbohydrate from biomass is the most abundant sustainable bioresource, where terrestrial plants fix CO_2 by using intermittent low energy density solar energy (~170 W/m^2) through photosynthesis. Although plants have low photosynthesis efficiencies of ~0.3% (*2*), the yearly chemical energy (phytobiomass) produced by photosynthesis is nearly six-fold of the total world's energy consumption (*3*). Utilization of a small fraction of renewable biomass carbohydrate would be sufficient to replace crude oil, especially in the transport sector.

Although prices of different energy sources or carriers range greatly, they in an increasing order are carbohydrates ($10.6 per GJ, $0.18 per kg), electricity ($16.7 per GJ, $0.04 per kWH), methanol ($17.8 per GJ, $0.35 per kg), gasoline ($17.6 per GJ, $2.5 per gallon), diesel ($19.5 per GJ, $2.7 per gallon), ethanol ($22.1 per GJ, $2 per gallon), hydrogen ($25.0 per GJ, $3 per kg), and biodiesel ($27.4 per GJ, $3.5 per gallon). Therefore, it is economically appealing to generate relatively high value hydrogen from low-cost biomass carbohydrates.

Hydrogen Production

Hydrogen can be produced from biomass carbohydrate by chemical catalysis featuring harsh reaction conditions, biocatalysis featuring modest reaction conditions, and their combinations (Fig. 1).

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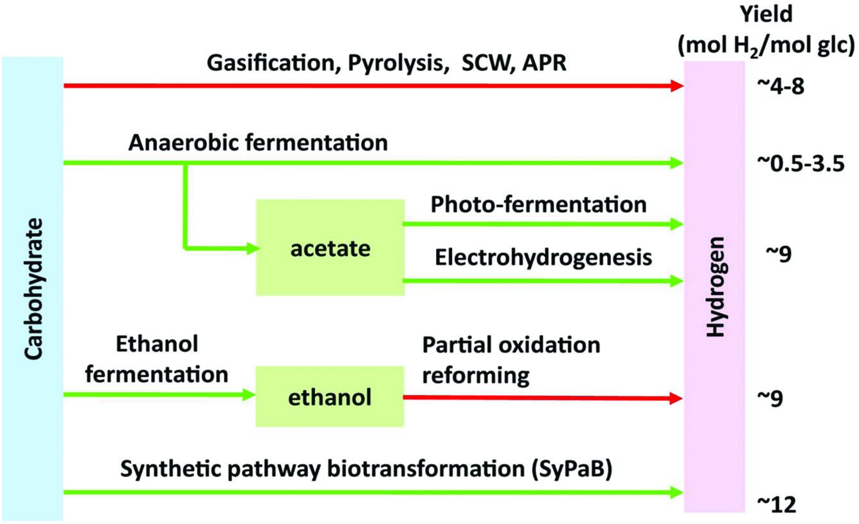
Yield
(mol H_2/mol glc)
Gasification, Pyrolysis, SCW, APR
~4-8
Anaerobic fermentation
~0.5-3.5
Carbohydrate
acetate
Photo-fermentation
Electrohydrogenesis
Hydrogen
~9
Ethanol
fermentation
ethanol
Partial oxidation
reforming
~9
Synthetic pathway biotransformation (SyPaB)
~12

Figure 1. Comparison of different approaches to generating hydrogen from biomass carbohydrates.

Chemical Catalysis

Chemical catalysis for hydrogen generation can be classified based on a decreasing temperature order – gasification, pyrolysis, gasification in critical water, and aqueous phase reforming (APR). All of them suffer from relatively low yields of hydrogen (e.g., ~ 6-8 hydrogen per glucose).

Gasification is usually operated at the temperature above 1000 K and in the presence of oxygen and/or water. Coupled by water-gas shift reaction, gasification is more favorable for the production of hydrogen than pyrolysis (*8*). The gas generated from biomass gasification generally contain contaminants, such as particulates, ashes, alkali compounds, nitrogen-containing components, sulfur, and low-molecular-weight hydrocarbons (e.g. methane and ethane) (*9*). The need to remove the contaminants from the gas steam depends on the end use of the gas. Several reports and reviews are available (*9*–*12*). The integrated process containing an air-blown bubbling fluidized bed gasifier, a steam reformer, and a water-gas-shift membrane reactor has been reported to completely decompose tar and produce ultrapure hydrogen through biomass gasification (*13*, *14*).

Pyrolysis is chemical decomposition of a condensed substance induced by heat without oxygen (*9*). Pyrolysis of coal and biomass (primarily wood) was popular for producing fuel-related gas and smokeless solid fuel, e.g. charcoal, from 1700s to early 1900s (*15*). Now it is applied to convert biomass into syngas, to produce coke from coal, and to treat hazardous wastes. The product yield and composition through biomass pyrolysis depend on reaction temperature, heating rate, and particle size of biomass (*16*, *17*). High temperatures promote gas production, while lower temperatures favor the formation of char and tar (or heavy oil) (*18*). In the rapid heating experiments, the rapid removal of volatiles

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from the reactor prevents the formation of secondary char and attributes to the higher volatile yield and lower char yield.

Gasification in supercritical water (SCW) is conducted at pressures and temperature above the critical point (221 Bar and 647 K) of water to supercritical conditions. Different from regular gasification that works on biomass with moisture contents of ~10-20% (*19*), SCW can gasify wet biomass with a moisture content of more than 35%. It is promising to gasify wet biomass because of the high gasification (100% conversion) and hydrogen ratios (50 vol %) (*20*, *21*).

Aqueous-phase reforming (APR) is reforming under relatively high temperature (400-550 K) and high pressure (50-70 bar), where the reactions occurs in an aqueous phase (*22*). In 2002, Dumesic *et al.* firstly demonstrated that hydrogen can be produced from biomass-derived carbohydrates at temperatures near 500 K in a single-reactor by using a platinum-based catalyst (*23*). The advantages of APR over vapor-phase reforming of oxygenated carbohydrates are (i) favorable water-gas shift reaction under the reaction conditions, generating hydrogen with low levels of CO (around 100 ppm); (ii) major energy savings from separation of gas hydrogen and aqueous water; (iii) reduction in undesirable decomposition reactions when carbohydrates are heated under modest conditions; and (iv) a better control of the performance of the catalytic process. The disadvantage is leaching and destability of catalyst components into the aqueous phase. Although APR process may be attractive to produce H_2 from carbohydrates, hydrogen formed had very low yields (1.05-1.41 mmol per gram of carbohydrates) to date owing to formation of coke and by-products (*24*).

Biological Catalysis

Biological catalysis is mediated by microorganisms or enzymes at ambient temperature and around atmospheric pressure. Biocatalysis has advantage over catalysis, such as higher selectivity, lower energy input, and less costly bioreactor (*25*, *26*). But it suffers from lower reaction rates. For high-water content organic resources, such as wastewater, sewage sludge, etc., biological approaches mediated by microorganisms is the only cost-efficient way to produce hydrogen (*27*). Hydrogen can be produced by dark (anaerobic fermentation), light fermentation, their combination, microbial electrohydrogenesis, and cell-free synthetic enzymatic pathway biotransformation (SyPaB).

Most of biohydrogen or methane in nature is produced from carbohydrates or their metabolic products by anaerobic microorganisms without light -- dark fermentation. In principle, one mole of glucose can produce four moles of hydrogen and two mole of acetate or two hydrogen and one butyrate through the mixed acid pathway, called the Thauer limit (*28*). In practice, hydrogen yields are much lower than this theoretical yield (4 H_2/glucose). High H_2 yields are usually associated with acetate production, and low yields are related with the production of propionate or other reduced end products, like alcohols and lactic acid (*29*). *Enterobacter*, *Bacillus*, and *Clostridium spp. (e.g. Clostridium pasteaurianum*, *C. butyricum*, and *C. beijerinki)* are well-known species to produce biohydrogen (*30*).

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Due to poor hydrogen yields, dark-fermentation is far from practical solutions to hydrogen production based on relatively pure carbohydrate but is operative to produce hydrogen from waste water (*31*). In order to achieve the Thauer limit (4 mol H_2 per mol glucose), hydrogen production should be conducted under very low partial pressures of H_2 with very slow rates (*25*, *32*). In order to get close to the theoretical yield, intensive efforts have been made through process optimization, reactor design, and metabolic engineering (*25*, *33*, *34*).

To increase overall hydrogen yields, acetate can be converted to hydrogen by electrohydrogenesis or photo-fermentation (Fig. 1). Since hydrogen generation from acetate is thermodynamically unfavorable, extra energy needs input. Electrohydrogenesis is a process in which exoelectrogenic bacteria generate protons and electrons in modified microbial fuel cells by using chemical energy in acetate plus a small amount of electric energy (*35*). Experimental results show that hydrogen production is dependent on the voltage supplied. The minimum voltage for hydrogenesis is 0.11 V at the cathode based on the thermodynamic analysis. The yields are 2.01-3.95 mol H_2 per mol acetic acid by applying the voltages of 0.2 to 0.8 V. The overall yields are 8.55 mol H_2 per mol glucose on glucose and 8.20 mol H_2 per mol hexose equivalent of cellulose. The corresponding hydrogen production rates were 2.29 mmol/L/h and 0.20 mmol/L/h for glucose and cellulose, respectively (*35*). It is worth noting that capital investment for microbial fuel cells may be too high to prevent its scale-up as compared to mature anaerobic digestion (*36*).

Alternative, photosynthetic bacteria can utilize organic acids plus solar energy for H_2 production (*37*, *38*), but very slow hydrogen generation rates (e.g., one order of magnitude lower than those of dark fermentation) and high hidden costs in hydrogen collection from a non-point (large area) source may prevent it from potential applications. Integration of multiple processes lead to more challenges in reactor engineering, system design, process control, and operation and maintenance (*39*). For example, in order to obtain a maximum utilization of the substrate, the system should be well-controlled to provide optimum media composition and environmental conditions for the two microbial components of the process (*40–42*) . For example, ammonia concentration and C/N ratio in the effluent from the first stage should not inhibit the hydrogen production in the second stage (*41*, *43*). Dilution and neutralization is thus required before photo fermentation to adjust the organic acid concentration and the pH level (*42*). Other challenges include i) adjusting photosynthetic and respiration capacity ratio, ii) co-culture balance, and iii) pretreatment of cell biomass from dark fermentation for photo fermentation (*39*).

Cell-free synthetic enzymatic pathway biotransformation (SyPaB), a new direction of synthetic biology or *in vitro* metabolic engineering, is implementation of complicated biological reaction network by *in vitro* assembling a number of enzymes and coenzymes (*6*, *7*, *44*). To break the Thauer limit for hydrogen-producing microorganisms, the synthetic enzymatic pathways have been designed to produce 12 moles of hydrogen per mole of glucose equivalent of glucan (starch or cellulose) and water (Fig. 2a) (*45–47*). The reconstituted non-natural catabolic pathways degrade polysaccharides initially to glucose 1-phosphate (g1p) and eventually to CO_2, split water and finally release the

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chemical energy in the form of hydrogen gas. These processes are like catabolism where water rather than oxygen works as an oxidant receiving electrons and generates hydrogen and CO_2 (*46*).

The pathways contain five sub-modules: (i) polysaccharide or oligosaccharide conversion to glucose-1-phosphate (g1p) catalyzed by phosphorylases, (ii) glucose-6-phosphate (g6p) generation from g1p catalyzed by phosphoglucomutase, (iii) NADPH production catalyzed by two dehydrogenases of the oxidative phase of the pentose phosphate pathway (PPP), (iv) g6p regeneration from ribulose-5-phosphate catalyzed by the eight enzymes of the non-oxidative phase of PPP, glycolysis and gluconeogenesis pathways, and (v) hydrogen generation from NADPH catalyzed by hydrogenase. The overall carbohydrate-to-hydrogen reaction can be summarized as

$$C_6H_{10}O_5\ (aq) + 7\ H_2O\ (l) \rightarrow 12\ H_2\ (g) + 6\ CO_2\ (g) \qquad [1]$$

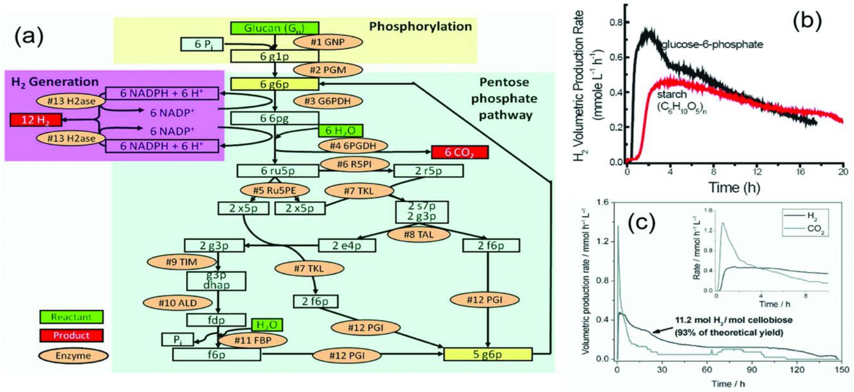
(a)
Phosphorylation
Glucan (Gn)
6 Pi
#1 GNP
6 g1p
#2 PGM
6 g6p
H2 Generation
#13 H2ase
6 NADPH + 6 H+
6 NADP+
12 H2
#3 G6PDH
6 6pg
6 H2O
#4 6PGDH
6 CO2
Pentose phosphate pathway
6 ru5p
#6 R5PI
2 r5p
#5 Ru5PE
#7 TKL
2 x5p
2 s7p
2 g3p
#8 TAL
2 g3p
2 e4p
2 f6p
#9 TIM
g3p
dhap
#10 ALD
#7 TKL
2 f6p
#12 PGI
fdp
H2O
Pi
#11 FBP
f6p
5 g6p
Reactant
Product
Enzyme
(b)
H2 Volumetric Production Rate (mmole L-1 h-1)
glucose-6-phosphate
starch (C6H10O5)n
Time (h)
(c)
Volumetric production rate / mmol h-1 L-1
11.2 mol H2 / mol cellobiose (93% of theoretical yield)
Time / h
H2
CO2
Rate / mmol h-1 L-1
Time / h

Figure 2. The cell-free synthetic pathway for high-yield hydrogen generation (a), high-yield generation of hydrogen from starch (b) (47) or soluble cellodextrin (c) (45). The enzymes are: GNP, glucan phosphorylase; PGM, phosphoglucomutase; G6PDH, G-6-P dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; R5PI, phosphoribose isomerase; Ru5PE, ribulose 5-phosphate epimerase; TKL, transketolase; TAL, transaldolase; TIM, triose phosphate isomerase; ALD, aldolase; FBP, fructose-1, 6-bisphosphatase; PGI, phosphoglucose isomerase; and H2ase, hydrogenase. The metabolites and chemicals are: g1p, glucose-1-phosphate; g6p, glucose-6-phosphate; 6pg, 6-phosphogluconate; ru5p, ribulose-5-phosphate; x5p, xylulose-5-phosphate; r5p, ribose-5-phosphate; s7p, sedoheptulose-7-phosphate; g3p, glyceraldehyde-3-phosphate; e4p, erythrose-4-phosphate; dhap, dihydroxacetone phosphate; fdp, fructose-1,6-diphosphate; f6p, fructose-6-phosphate; and Pi, inorganic phosphate.

Thermodynamic analysis suggests that the overall reaction (Equation 1) is spontaneous (△G° = -48.9 kJ/mol) and endothermic (△H° = +596 kJ/mol) (*45*, *47*). This enzymatic reaction is among rare entropy-driven chemical reactions because two final products are gaseous under experimental conditions (<100°C and ~1 atm) (*45*). Great increases in the entropy from aqueous to gas phases enable the negative-enthalpy reactions to occur. To our limited knowledge, the reactions (Equation 1) may be the first chemical reaction that can generate hydrogen energy by absorbing waste heat.

We have demonstrated the feasibility of high-yield spontaneous generation of hydrogen from starch or cellulosic materials and water in batch reactions (Fig. b&c) (*45*, *47*). It is expected that 100% product yield (i.e., 12 H_2/glucose equivalent) is achieved in a continuous reactor. During the past three years, we have increased the reaction rates by nearly 20-fold through optimization of rate-limiting enzyme loadings, increasing substrate concentrations from 2 to 8 mM, and elevating reaction temperatures slightly from 30 to 32°C. The current production rate of H_2 is 3.92 mmol H_2/L/h (*45*), higher than those of photobiological systems and comparable to those reported in dark fermentations and electrohydrogensis (*4*, *35*).

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A Combination of Catalysis and Biocatalysis

Another carbohydrate-to-hydrogen production process is a hybrid of biological and chemical catalysis, both of which have high selectivity under their conditions. First, polysaccharides are hydrolyzed to glucose by using hydrolases, such as amylases and cellulases (*48*, *49*). Then ethanol-producing yeasts or bacteria can convert glucose to ethanol with nearly theoretical yields (i.e., two ethanol per glucose) (*48–51*). Alternatively, consolidated bioprocessing (CBP) microorganisms that can produce cellulase, hydrolyze cellulose, and ferment ethanol can convert solid cellulose to ethanol in a single step (*50*, *52–55*). Second, ethanol after distillation can be converted to hydrogen by partial oxidation reforming (*56*, *57*). But the reformed product still contain a small amount of CO, which must be removed before entering proton exchange membrane fuel cells. The overall theoretical hydrogen yield of this hybrid is 10 H_2 per glucose. But considering energy conversion losses (e.g., carbohydrate use for cell mass synthesis, and partial oxidation reforming), the practical hydrogen yield through this hybrid is approximately nine hydrogen per glucose.

Opportunities and Obstacles of SyPaB

Among different carbohydrate-to-hydrogen technologies, SyPaB is the only way that can produce nearly theoretical hydrogen yield. SyPaB is a new direction of synthetic biology. Synthetic biology applies engineering principles (e.g., design, extraction, and standardization) and combines science (biology and chemistry) for designing and building novel biological functions and systems that function unnaturally or function much better than natural counterparts. Synthetic biology can also be interpreted as the engineering-driven building of increasingly complicated biological entities (parts, devices, and systems) from simple and basic building blocks. The design principles of cell-free synthetic biology are so clear that we are able to assemble a new system much more easily than to modify a living system without constraints from cellular viability, complexity, physiology, and the presence of membranes and /or walls (*44*).

Microbial fermentation is different from SyPaB in that microbes can duplicate themselves but enzymes cannot. Since microbes can self-duplicate and self-repair, the costs associated with microbe production are low and there is no cost for enzyme separation, stabilization, and co-factors. In contrast, SyPaB requires production, purification, and stabilization of enzymes based on microbial fermentation as well as the addition of costly co-enzymes.

Figure 3 shows comparison of microbial fermentations and SyPaB for biofuel production. A typical one-step microbial fermentation where the formation of product is associated with cell growth (Fig. 3a), for example, production of membrane lipids from carbohydrate (*58*). When the formation of desired product is not dissociated with cell growth, two-step microbial fermentation is usually conducted. For example, ethanol fermentation can be carried out in two steps: first, cell growth under aerobic conditions; second, ethanol production under anaerobic conditions. SyPaB may be regarded as atypical two-step fermentation. In the first step, several mesophilic microorganisms (e.g., *E. coli*) are cultivated separately for producing recombinant high-yield thermostable enzymes. After cell lysis, thermostable enzymes can be purified by low-cost approaches, such as, simple adsorption (*59*, *60*) or heat precipitation (*61*) because most of the *E. coli* cellular proteins, which are not stable at high temperature, can be precipitated by heat treatment (*44*). In the second step, numerous purified enzymes are reconstituted for high-speed biotransformation. If necessary, thermostable enzymes may be immobilized for higher turn-over number (TTN, mol product/mol enzyme) and/or better product/enzyme separation. Because the enzymes have several orders of magnitude total turn-over number higher than those of microbes (*6*, *7*), microbial fermentation-SyPaB would show economically advantageous over two-step microbial fermentation in long-term operation.

Production costs for hydrogen through SyPaB are mainly based on three major cost components – carbohydrate, enzymes, and coenzyme (NAD). Figure 4a shows the effects of the costs of enzyme ($40 or $4000/kg enzyme) on hydrogen production costs. The cost decreases rapidly with increasing total turn-over number (TTN, mol product per mol of enzyme) of the enzymes in SyPaB, and then levels off when all enzymes regardless of their production costs have TTN values of more than 10^{8-9}. When all enzymes have TTN values of 3×10^7 and each one has production costs of $~40/kg, hydrogen production cost is anticipated to be $1.87 per kg H_2, where carbohydrate ($0.18/kg carbohydrate) accounts for approximately 66% of hydrogen production costs. When TTN values of the enzymes are further enhanced to 10^8 or 10^9, the ultimate cost of hydrogen would be as low as $1.30 per kg H_2. The above hydrogen production by SyPaB would be lower than its generation from natural gas (e.g., $2.00-2.70 per kg hydrogen).

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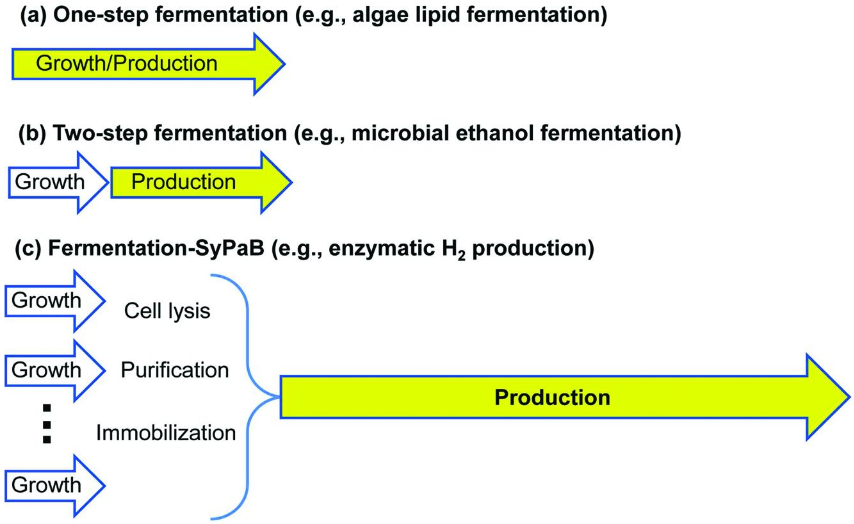
(a) One-step fermentation (e.g., algae lipid fermentation)
Growth/Production
(b) Two-step fermentation (e.g., microbial ethanol fermentation)
Growth
Production
(c) Fermentation-SyPaB (e.g., enzymatic H2 production)
Growth
Cell lysis
Growth
Purification
Immobilization
Growth
Production

Figure 3. Comparison of microbial fermentation and cell-free synthetic pathway biotransformation.

The above economical analysis is based on two important assumptions: (i) enzyme production costs and (ii) enzyme TTN values. Typical industrial enzymes have production costs from $~5-40/kg dry protein weight, for example, protease, cellulase, amylase and so on. Fig. 4b shows typical TTN values of the enzymes in industrial applications and obtained in our laboratory. Very low TTN values for cellulase result in poor economical viability of biomass saccharification (*6*). Amylase has much higher TTN values than cellulase so that the enzymes costs are much more lower in starch ethanol biorefineries. For fructose production from glucose, ultra-stable immobilized themophilic glucose isomerase leads to enzyme costs to minimal levels. In our laboratory, we have obtained three thermostable enzymes with TTN values of more than 10^7, for example, *Clostridium thermocellum* phosphoglucomutase (*62*), *Thermotoga maritima* fructose-1,6-bisphosphatase (*63*), and *T. maritima* 6-phosphogluconate dehydrogenase (*61*). It is found that free *C. thermocellum* phosphoglucose isomerase has low TTN values but it becomes ultra-stable (more than 10^9) after simple immobilization through adsorption on cellulose surface by using cellulose-binding module (*64*). Clearly, it is highly operative to obtain numerous high-TTN non-membrane enzymes suitable for biocommodity production by using SyPaB.

With developments in (i) engineered oxidoreductases that can use biomimetic NAD factors (*65–67*) and (ii) stable enzymes as building blocks of SyPaB (*61–64*, *68*), we estimate that hydrogen production costs may decrease to ~$1.30 per kg of hydrogen (Fig. 4), where carbohydrate accounts for ~95% of its production costs.

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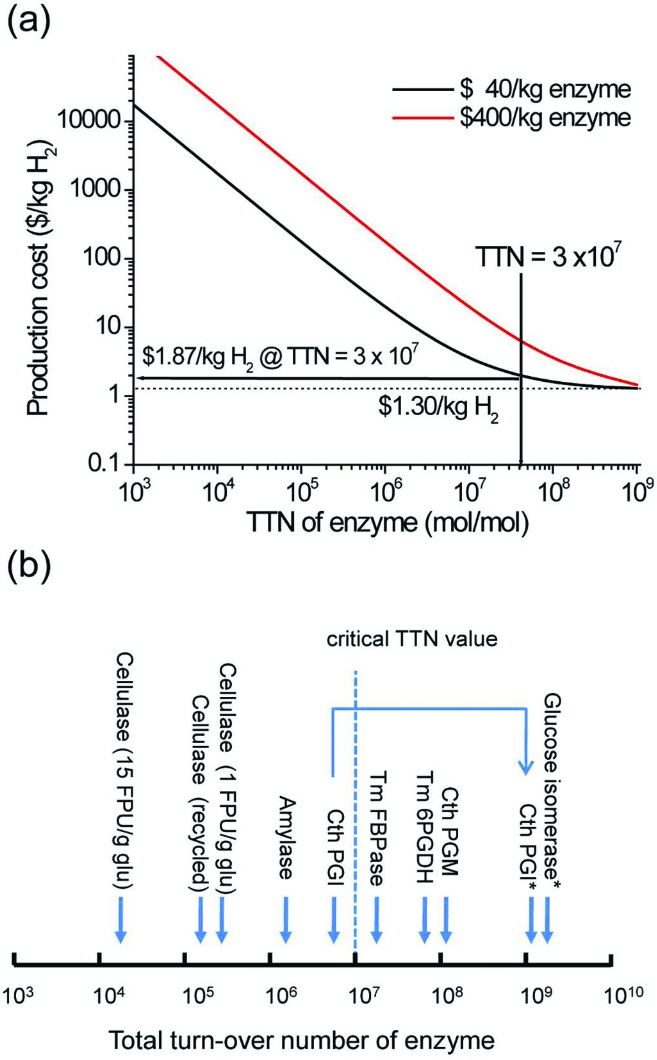

Figure 4. Hydrogen production cost analysis (a) and typical total turn-over number (TTN) values of enzymes.

Concluding Remarks

Hydrogen production costs are highly based on its yield on carbohydrate. The USA DOE report has set a bottom conversion goal at 50% efficiency (e.g., 6 H_2/glucose) for hydrogen production from biomass (*69*). This mini-review presents different yields for different carbohydrate-to-hydrogen technologies (Fig. 1). Several new technologies have been achieved for producing more than 8 mol H_2 per mol hexose equivalent. Among them, SyPaB is highly promising because of its highest yield (plus extra hydrogen generation by utilizing waste heat),

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modest reaction condition, acceptable reaction rates, and low-cost bioreformers or bioreactors because in long terms thermodynamics (efficiency) decides economics (i.e. cost) (*58*, *70*). The obstacles to commercial hydrogen production by SyPaB – (i) a lack of thermostable enzymes and (ii) modifying oxidoreductases that can work on low-cost and stable biomimetic cofactors – are being addressed through international collaboration. In the past, strong motivations have driven to discover and engineer thermostable enzymes with obvious applications, such as DNA polymerase, amylase, glucose isomerase, cellulase, and so on. When the concept of SyPaB is accepted and more stable enzyme building blocks and stable biomimetic cofactor analogues are available, cell-free SyPaB would compete with microbial fermentations for the production of low-value biocommodities (Fig. 3).

In addition to high-yield hydrogen generation, this carbohydrate-to-hydrogen technology by SyPaB would address more challenges associated with the hydrogen economy, such as, storage, safety, distribution, and infrastructure of the hydrogen economy (*3*, *44*, *47*). But in short terms, hydrogen production through catalysis based on lignocellulosic biomass or dark fermentation based on waste may be more practical.

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References

1. Kerr, R. A; Service, R. F. *Science* **2005**, *309*, 101.
2. Zhang, Y.-H. P. *J. Ind. Microbiol. Biotechnol.* **2008**, *35* (5), 367–375.
3. Zhang, Y.-H. P. *Energy Environ. Sci.* **2009**, *2*, 272–282.
4. Das, D.; Veziroglu, T. N. *Int. J. Hydrogen Energy* **2001**, *26*, 13–28.
5. Turner, J. A. *Science* **2004**, *305*, 972–974.
6. Zhang, Y.-H. P. *Biotechnol. Bioeng.* **2010**, *105*, 663–677.
7. Zhang, Y.-H. P.; Sun, J.-B.; Zhong, J.-J. *Curr. Opin. Biotechnol.* **2010**, *21*, 663–669.
8. Ni, M.; Leung, D. Y. C.; Leung, M. K. H.; Sumathy, K. *Fuel Proc. Technol.* **2006**, *87*, 461–472.
9. Rezaiyan, J.; Cheremisinoff, N. P. *Gasification Technologies: A Primer for engineers and Scientists*. CRC Press. Boca Raton, FL; 2005; pp119−145.
10. Stevens, D. J. *Hot Gas Conditioning: Recent Progress with Larger-Scale Biomass Gasification Systems*; National Renewable Energy Laboratory: Golden, CO, 2001.
11. Cummer, K. R.; Brown, R. C. *Biomass Bioenergy* **2002**, *23*, 113–128.
12. Hallgren, A. L. *Hot Gas Cleanup in Biomass Gasification: Review of Activities within EC Sponsored R&D Programs*, 2010 National Meeting

Downloaded by DUKE UNIV on June 16, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch008

of the American Chemical Society, Fuels: Symposium on Direct Coal Liquefaction, Aug. 20−25, 1995, Chicago, IL.

13. Ji, P.; Feng, W.; Chen, B. *Chem. Eng. Sci.* **2009**, *64*, 582–592.
14. Lin, S.; Harada, M.; Suzuki, Y.; Hatano, H. *Energy Convers. Manag.* **2005**, *46*, 869–880.
15. Klass, D. L. *Biomass for Renewable Energy, Fuels, and Chemicals*; Academic Press: New York, 1998.
16. Zanzi, R.; Sjostrom, K.; Bjornbom, E. *Biomass Bioenergy* **2002**, *23*, 357–366.
17. Probstein, R. F.; Hicks, R. E. *Synthetic Fuels*; Dover Publications: Mineola, NY, 2006.
18. Klass, D. L.; Emert, G. H. *Fuels from Biomass and Wastes*; Ann Arbor Science Publishers: Ann Arbor, MI, 1981.
19. Rezaiyan, J.; Cheremisinoff, N. P. Biogasification. In *Gasification Technologies: A Primer for Engineers and Scientists*; CRC Press: Boca Raton, FL, 2005; p119−145.
20. Matsumura, Y.; et al. *Biomass Bioenergy* **2005**, *29* (4), 269–292.
21. Huber, G. W.; Iborra, S.; Corma, A. *Chem. Rev.* **2006**, *106* (9), 4044–4098.
22. Chheda, J.; Huber, G.; Dumesic, J. *Angew. Chem., Int. Ed.* **2007**, *46*, 7164–7183.
23. Cortright, R. D.; Davda, R. R.; Dumesic, J. A. *Nature* **2002**, *418*, 964–967.
24. Valenzuela, M. B.; Jones, C. W.; Agrawal, P. K. *Energy Fuels* **2006**, *20*, 1744–1752.
25. Nath, K.; Das, D. *Appl. Microbiol. Biotechnol.* **2004**, *65* (5), 520–529.
26. Manish, S.; Banerjee, R. *Int. J. Hydrogen Energy* **2008**, *33*, 279–286.
27. Kotay, S. M.; Das, D. *Int. J. Hydrogen Energy* **2008**, *33*, 258–263.
28. Thauer, K.; Jungermann, K.; Decker, K. *Bacteriol. Rev.* **1977**, *41*, 100–180.
29. Vavilin, V. A.; Rytow, S. V.; Lokshina, L. Y. *Biores. Technol.* **1995**, *54*, 171–177.
30. Hawkes, F. R.; Dinsdale, R.; Hawkes, D. L.; Hussy, I. *Int. J. Hydrogen Energy* **2002**, *27*, 1339–1347.
31. Benemann, J. *Nat. Biotechnol.* **1996**, *14*, 1101–1103.
32. Hallenbeck, P. C.; Benemann, J. R. *Int. J. Hydrogen Energy* **2002**, *27*, 1185–1193.
33. Maeda, T.; Sanchez-Torres, V.; Wood, T. K. *Microb. Biotechnol.* **2008**, *1* (1), 30–39.
34. Veit, A.; Akhtar, M. K.; Mizutani, T.; Jones, P. R. *Microb. Biotechnol.* **2008**, *1*, 382–394.
35. Cheng, S.; Logan, B. E. *Proc. Nat. Acad. Sci. USA* **2007**, *104*, 18871–18873.
36. Foley, J. M.; Rozendal, R. A.; Hertle, C. K.; Lant, P. A.; Rabaey, K. *Environ. Sci. Technol.* **2010**, *44*, 3629–3637.
37. Fascetti, E.; Todini, O. *Appl. Microbiol. Biotechnol.* **1995**, *44*, 300–305.
38. Ueno, Y.; Tatara, M.; Fukui, H.; Makiuchi, T.; Goto, M.; Sode, K. *Biores. Technol.* **2007**, *98*, 1861–1865.
39. Holladay, J. D.; Hu, J.; King, D. L.; Wang, Y. *Catal. Today* **2009**, *139*, 244–260.

Downloaded by DUKE UNIV on June 16, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch008

40. Yokoi, H.; Saitsu, A.; Uchida, H.; Hirose, J.; Hayashi, S.; Takasaki, Y. *J. Biosci. Bioeng.* **2001**, *91*, 58–63.
41. Fascetti, E.; D'Addario, E.; Todini, O.; Robertiello, A. *Int. J. Hydrogen Energy* **1998**, *23*, 753–760.
42. Kapdan, I. K.; Kargi, F. *Enzyme Microb. Technol.* **2006**, *38*, 569–582.
43. Lee, C.-M.; Chen, P.-C.; Wang, C.-C.; Tung, Y.-C. *Int. J. Hydrogen Energy* **2002**, *27*, 1309–1313.
44. Zhang, Y.-H. P.; Mielenz, J. R. *Energies* **2011**, *4*, 254–275.
45. Ye, X.; Wang, Y.; Hopkins, R. C.; Adams, M. W. W.; Evans, B. R.; Mielenz, J. R.; Zhang, Y.-H. P. *ChemSusChem* **2009**, *2*, 149–152.
46. Zhang, Y.-H. P. *Microbe* **2009**, *4*, 560–565.
47. Zhang, Y.-H. P.; Evans, B. R.; Mielenz, J. R.; Hopkins, R. C.; Adams, M. W. W. *PLoS One* **2007**, *2*, e456.
48. Zhang, Y.-H. P.; Lynd, L. R. *Biotechnol. Bioeng.* **2004**, *88*, 797–824.
49. Zhang, Y.-H. P.; Himmel, M.; Mielenz, J. R. *Biotechnol. Adv.* **2006**, *24*, 452–481.
50. Lynd, L. R.; Weimer, P. J.; van Zyl, W. H.; Pretorius, I. S. *Microbiol. Mol. Biol. Rev.* **2002**, *66*, 506–577.
51. Zhang, Y.-H. P.; Lynd, L. R. *Biotechnol. Bioeng.* **2006**, *94*, 888–898.
52. Zhang, Y.-H. P.; Lynd, L. R. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 7321–7325.
53. Lu, Y.; Zhang, Y.-H. P.; Lynd, L. R. *Proc. Nat. Acad. Sci. USA* **2006**, *103* (44), 16165–16169.
54. Zhang, Y.-H. P.; Lynd, L. R. *J. Bacteriol.* **2005**, *187*, 99–106.
55. Zhang, X.-Z.; Zhang, Y.-H. P. *Eng. Life Sci.* **2010**, *10*, 398–406.
56. Deluga, G. A.; Salge, J. R.; Schmidt, L. D.; Verykios, X. E. *Science* **2004**, *303*, 993–997.
57. Haryanto, A.; Fernando, S.; Murali, N.; Adhikari, S. *Energy Fuels* **2005**, *19*, 2098–2106.
58. Huang, W. D.; Zhang, Y.-H. P. *Energy Environ. Sci.*, **2011**, Epub, DOI: 10.1039/C0EE00069H.
59. Hong, J.; Wang, Y.; Ye, X; Zhang, Y.-H. P. *J. Chromatogr., A.* **2008**, *1194*, 150–154.
60. Hong, J.; Ye, X; Wang, Y.; Zhang, Y.-H. P. *Anal. Chim. Acta.* **2008**, *621*, 193–199.
61. Wang, Y.; Zhang, Y.-H. P. *Microb. Cell Fact.* **2009**, *8*, 30.
62. Wang, Y.; Zhang, Y.-H. P. *J. Appl. Microbiol.* **2010**, *108*, 39–46.
63. Myung, S.; Wang, Y. R.; Zhang, Y.-H. P. *Process Biochem.* **2010**, *45*, 1882–1887.
64. Myung, S.; Zhang, X.-Z.; Zhang, Y.-H. P. *Biotechnol. Prog.* **2011**, accepted.
65. Fish, R. H.; Kerr, J. B.; Lo, H. C. Agents for Replacement of NAD^+/NADH System in Enzymatic Reaction, U.S. Patent 6,716,596 B2, 2004.
66. Lo, H. C.; Fish, R. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 478–481.
67. Ryan, J. D.; Fish, R. H.; Clark, D. S. *ChemBioChem* **2008**, *9*, 2579–2582.
68. Ye, X.; Rollin, J.; Zhang, Y.-H. P. *J. Mol. Catal. B: Enzym.* **2010**, *65*, 110–116.

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

69. U.S. Department of Energy. *A Multiyear Plan for the Hydrogen R&D Program, Rationale, Structure and Technology Roadmaps*, 1999.
70. Smil, V. *Energy Transitions: History, Requirements, Prospects*; ABC-CLIO, LLC: Santa Barbara, CA, 2010.

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Chapter 9

Chemicals from Hemicelluloses: A Review

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Lignocellulosic biomass such as agricultural and forestry residues, and dedicated crops provides a low-cost and uniquely sustainable resource for production of organic fuels and chemicals that can reduce greenhouse gas emissions, enhance energy, dispose of problematic solid waste, and improve air quality. Effective utilization of biomass, hitherto underutilized, is gaining tremendous importance for the production of biobased fuels, chemicals and polymeric materials. Hemicelluloses rank second to cellulose in abundance in lignocellulosic biomass, comprising roughly one-fourth to one-third. They represent a type of hetero-polysaccharides with complex structures containing glucose, xylose, mannose, galactose, arabinose, glucuronic acid, and galacturonic acid in various amounts depending on the sources. In this paper, the structure of hemicelluloses and the bioconversion and chemical transformation of hemicelluloses for chemicals are reviewed. The main chemicals from the hemicelluloses, ethanol, xylitol, 2,3-butanediol, furfural, 5-hydroxymethylfurfural, and levulinic acid will be discussed.

Introduction

In the twentieth century, the major research emphasis was given for the development of petroleum, coal, and natural gas based refinery to exploit the cheaply available fossil feed stock. These feedstocks are used in industry

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to produce multiple products such as fuel, fine chemicals, pharmaceuticals, detergents, synthetic fiber, plastics, pesticides, fertilizers, lubricants, solvent, waxes, coke, asphalt, etc (*1–3*). Nowadays, a great fraction of worldwide energy carriers and material products come from fossil fuel refinery. Due to the on-going price increase in fossil resources, their uncertain availability, and their environmental concerns, the feasibility of fossil resources exploitation are predicted to decrease in the near future (*4*). Consequently, these uncertainties have rekindled a strong interest in pursuing alternative and renewable energy spurces. Therefore, conversion of biomass/lignocellulosic materials is regarded as one of the most promising alternatives to fossil fuels for production of biofuels, biochemicals, and biomaterials (*5*).

Biomass is the world′s fourth largest energy source worldwide, following coal, oil and natural gas. About 170 billion tons of biomass are produced annually, but only 3% is used by human consumption (food and non-food) (*6*). In fact, there is an agreement that biomass production is sufficient to feed all people, to provide the population with the required organic materials and to make a contribution to our energy needs (*7*, *8*). Biomass for energy, especially biofuels, has positive attributes that contribute to a healthy environment and economy. Biomass utilization can reduce forest management costs, help mitigate climate change, reduce risks to life and property, and help provide a secure, and competitive energy source. Therefore, the replacement of petroleum with biomass as raw material for value-added biochemicals, bioenergy and biofuels is an effective option, which may lay the foundation for a stepwise shift of the current global economy toward a sustainable biobased economy (*9*).

Vegetable biomass is generated from CO_2 and H_2O using sunlight as the energy source, producing O_2 as a sub-product. The primary products formed are C_6- and C_5- sugars that form cellulose (by polymerization of glucose) and hemicelluloses (a polymer of mannose, arabinose, galactose, glucose and xylose). Lignin is the third component of biomass. It is a highly cross-linked polymer composed of substituted phenols, together with cellulose and hemicelluloses, and gives strength to plants (*10*). Hemicelluloses are the second most abundant plant renewable biopolymer after cellulose. An estimated annual production of hemicelluloses on the earth is in the range of 60 billion tons (*3*). They differ from cellulose in the main cell wall constituent, which is a highly uniform (1→4)-β-linked polyglucan. There are two types of sugars in the hemicelluloses, pentose (xylose and arabinose) and hexose (glucose, galactose, and mannose). The utilization of hemicellulosic sugars is essential for efficient conversion of lignocellulosic materials to fuel ethanol and other high value-added chemicals. There are two ways to transform hemicellulosic sugars into chemicals: one is bioconversion (fermentation) process, and the other is the chemical transformation. In this present paper, a brief review on the hemicelluloses structure and the bioconversion and chemical transformation of hemicelluloses is presented. The main chemicals derived from the hemicelluloses will be discussed.

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The Structure of Hemicelluloses

Hemicelluloses rank second to cellulose in abundance in plant cell walls, comparing roughly one-fourth to one-third of most plant materials, and the amount will vary according to the particular plant species, such as maize stems (28.0%), barley straw (34.9%), wheat straw (38.8%), and rye straw (36.9%) (*11*). Hemicelluloses are heterogeneous polymers of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acids. In plant cell walls there are large amounts of hemicelluloses with a wide variation in content and chemical structure. Typical composition of hemicelluloses is presented in Table 1. In hardwoods, the principal hemicellulose is an *O*-acetyl-4-*O*-methylglucuronoxylans with amounts between 15% and 30% depending on the species, except for small portions of glucomannan (2–5%). The percentage of acetyl groups ranges between 8% and 17% of total xylan, corresponding, in average, to 3.5–7.0 acetyl groups per 10 xylose units (*12*). The 4-*O*-methylglucuronic acid side groups are more resistant to acids than the xylopyranose and acetyl groups. Besides these main structural units, glucuronoxylans (GX) may also contain small amounts of L-rhamnose and galacturonic acid. The average degree of polymerization (DP) of GX is in the range of 100–200 (*13*). However, the main hemicelluloses of softwoods are an *O*-acetyl-galactoglucomannan with contents between 10% and 25%. Here the arabino-4-*O*-methyl-glucuronoxylan exits in minor quantities of around 5-10%. Gramineae such as cereal straws, the main hemicelluloses is arabinoxylans (AX). AXs are similar to harwoods xylan but the amount of L-arabinose is higher. In AX, the linear β-(1→4)-D-xylopyranose backbone is substituted by α-L-arabinofuranosyl units in the positions 2-*O* and/or 3-*O* and by α-D-glucopyranosyl uronic unit or its 4-*O*-methyl derivative in the position 2-*O* (*14*, *15*). *O*-acetyl substituents may also occur (*16*, *17*). Bacon et al. (*18*) mentioned that cell walls of Gramineae plants account for 1-2% of the acetyl groups. Arabinofuranosyl residues of AX may also be esterified with hydroxycinnamic acids residues, e.g., ferulic and *p*-coumaric acids (*16*). Dimerization of esterified phenolic compounds may also lead to inter- and intra-molecular cross-links of xylan. The physical and/or covalent interactions with other cell wall constituents restrict the extractability of xylan. In addition, xyloglucans are quantitatively predominant hemicellulosic polysaccharide type in the primary cell walls of wood (mainly in hardwoods and less in softwoods) (*19*). It can also appear in small amounts in grasses (*20*). Xyloglucans consist of β-(1→4)-linked D-glucose backbone with 75% of these residues substituted at *O*-6 with D-xylose. L-arabinose and D-galactose residues can be attached to the xylose residues forming di-, or triglycosyl side chains. Also L-fucose has been detected attached to galactose residues at 2-*O*. Galactose residues can contain *O*-linked acetyl groups (*21*, *22*). Scheller et al. (*20*) grouped the hemicelluloses into xyloglucan, xylans, mannans and glucomannans, and β-(1→3, 1→4)-glucans. β-(1→3, 1→4)-glucans are well known in grasses. These mixed linked glucans are dominated by cellotriosyl and cellotetrasyl units linked by β-(1→3) linkages, but longer β-(1→4)-linked segments also occur (*23*). The β-(1→3, 1→4)-glucans are mainly exist in Poales and a few other groups.

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The presence of lignin-hemicelluloses linkages was studied in detail from straw, grass and wood samples (*24*, *25*). It is commonly assumed that lignin is tightly linked to polysaccharides in the cell walls of plants by variously linkage types, such as ether linkage of hydroxyl group at the α-position of lignin side chain with alcoholic hydroxyl of sugar residue, and ester linkage of the cinnamic acid unit in lignin with the alcoholic OH of polysaccharides (*26*, *27*). Our previous studies found that the majority of lignin in cereal straw cell walls is directly linked to arabinose side chains of xylan by ether bonds (*28*). Another potential lignin-hemicelluloses linkage is an ester bond between the lignin and carboxyl (C-6) group of uronic acid residues (*29*). More importantly, it was reported that ferulic acid ether linkage to lignin formed a cross-link to hemicelluloses through an ester linkage (hemicelluloses-ester-ferulic acid-ether-lignin bridges) (*16*, *30*). In addition, there is evidence that, during the assembly, the synthesis and deposition of xylan is intimately linked with cellulose (*31*). Hemicelluloses have also been shown to be linked with secondary wall proteins. One example of specific secondary wall proteins located directly other than inferred from cDNA sequence, which have been shown in Loblolly pine (*31*), hydpocotyl of French bean (*32*) and in differentiating Zinnia cells (*33*, *34*).

Table 1. The main types of polysaccharides present in hemicelluloses (*20*, *25*, *36*)

Polysaccharide	*Biological origin*	*Amount*^{a}	*Units*			*DP*^{b}
			Backbone	*Side chains*	*linkages*	
Arabinogalactan	Softwoods	5-35	β-D-Galp	β-D-Galp α-L-Araf β-L-Arap	β-(1→6) α-(1→3) β-(1→3)	100-600
Xyloglucan	Hardwoods, softwoods, and grasses	2-25	β-D-Glcp β-D-Xylp	β-D-Xylp β-D-Galp α-L-Araf α-L-Fucp Acetyl	β-(1→4) α-(1→3) β-(1→2) α-(1→2) α-(1→2)	
Galactoglucomannan	Softwoods	10-25	β-D-Manp β-D-Glcp	β-D-Galp Acetyl	α-(1→6)	40-100

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Table 1. (Continued). The main types of polysaccharides present in hemicelluloses (*20*, *25*, *36*)

Polysaccharide	*Biological origin*	*Amount*[a]	*Units*			*DP*[b]
			Backbone	*Side chains*	*linkages*	
Glucomannan	Hardwoods	2-5	β-D-Manp β-D-Glcp			40-70
Glucuronoxylan	Hardwoods	15-30	β-D-Xylp	4-*O*-Me-α-D-GlcpA Acetyl	α-(1→2)	100-200
Arabinoglucuronoxylan	Grasses and softwoods	5-10	β-D-Xylp	4-*O*-Me-α-D-GlcpA α-L-Araf	α-(1→2) α-(1→3)	50-185
Glucuronoarabinoxylans	Grasses	15-30	β-D-Xylp	α-L-Araf 4-*O*-Me-α-D-GlcpA Acetyl	α-(1→2) α-(1→3)	
Homoxylans	Algae		β-D-Xylp[c]			
β-(1→3, 1→4)-glucan	Grasses	2-15	β-D-Glcp		β-(1→3) β-(1→4)	

[a] %, dry biomass. [b] Degree of polymerization. [c] May also present β-(1→3) linkages on the backbone.

Chemicals from Hemicelluloses

Increased scientific and commercial interest in the development of viable biorefinery strategies converting renewable raw materials into biofuels, platform chemicals and biodegradable materials is driven by environmental concerns, depleting petroleum resources, and public awareness (*5*). Depending on raw materials, technological processes, and products obtained, biorefinery platforms can be distinguished based on sugar (biochemical), syngas (thermochemical), biogas, or carbon-rich chains platforms. Sugar (biochemical) biorefinery platform is the promising emerging biorefinery platforms (*37*). Biochemical platform focuses on fermentation of sugars extracted from lignocellulosic feedstocks. After feedstock preparation (size reduction), conversion process involve three basic steps: 1) Convert biomass to sugar or other fermentation feedstock; 2)

Bioconversion of these biomass intermediates using biocatalysts; and 3) Process products to yield value-added chemicals, fuel-grade ethanol and other fuels, heat and/or electricity (*38*). In 2004, the US Department of Energy identifies twelve building block chemicals from about 300 candidates that can be produced from sugars via biological or chemical conversions. The twelve building blocks can be subsequently converted to a number of high-value bio-based chemicals or materials. Building block chemicals, as considered for this analysis, are molecules with multiple functional groups that possess the potential to be transformed into new families of useful molecules. The twelve sugar-based building blocks are 1,4-diacids (succinic, fumaric and malic), 2,5-furan dicarboxylic acid, 3-hydroxy propionic acid, aspartic acid, glucaric acid, glutamic acid, itaconic acid, levulinic acid, 3-hydroxybutyrolactone, glycerol, sorbitol, and xylitol/arabinitol (*39*). Recently, Bozell et al. (*40*) revisited "Top 10" high-value bio-based chemicals from biofrefinery carbohydrates. The updated building block chemicals are ethanol, furans, glycerol and derivatives, biohydrocarbons, lactic acid, succinic acid, hydroxypropionic acid/aldehyde, levulinic acid, sorbitol, and xylitol.

Biomass carbohydrates (cellulose and hemicelluloses) are the most abundant renewable resources available, and they are currently viewed as a feedstock for the Green Chemistry of the future (*41–43*). Hemicelluloses are being transformed into monomeric components with maximum yield and purity by chemical, enzyme-mediated, and thermal process (*44*). Hemicelluloses are relatively easily hydrolyzed by chemical or enzyme-mediated processes to their monomer components consisting of pentoses (xylose and arabionose) and hexoses (glucose, galactose, and mannose) (*35*). These hemicellulosic sugars can be transformed into ethanol, xylitol, 2,3-butanediol (2,3-BD), furfural, 5-hydroxymethylfurfural, levulinic acid, and other value-added chemicals. The use of hemicellulosic sugar as a primary substrate may produce multiple industrial significance compounds.

Chemicals from Hemicelluloses via Bioconversion

The carbohydrate fraction of the plant cell wall can be converted into fermentable monomeric sugars through acidic and enzymatic (hemicellulase/cellulase) reactions, which have been exploited to produce ethanol, xylitol, and 2, 3-butanediol via microbial fermentation processes (*45–47*). In recent years, much attention has been paid to bioconversion of hemicelluloses because of their practical applications in various agro-industrial processes, such as efficient conversion of hemicellulosic biomass to fuels and chemicals, delignification of paper pulp, digestibility enhancement of animal feed stock, clarification of juices, and improvement in the consistency of beer (*46–50*). In this section, enzymatic saccharification of xylans to fermentable sugars and the bioprocess for conversion to various chemicals are reviewed.

Enzymatic Hydrolysis

In order to foster the commercial production of chemicals and biofuels, bioconversion of the hemicelluloses into the fermentable sugars is essential. The most promising method for hydrolysis of polysaccharide to monomer sugars

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is by enzyme, i.e., cellulases and hemicellulases. Xylans are characterized by a β-(1→4)-linked-D-xylopyranosyl main chain which carries a variable number of side groups, such as L-arabinosyl, D-galactosyl, acetyl, feruloyl, *p*-coumaroyl and glucuronosyl residues. The frequency and composition of the branches are dependent on the xylan source (*51–53*). Owing to its heterogeneity and complexity, the complete hydrolysis of xylan needs the action of several hemicellulases (*52*, *54*, *55*). Total biodegradation of xylan requires endo-β-1,4-xylanase, β-xylosidase, and several accessory enzymes, such as α-L-arabinofuranosidase, α-glucuronidase, acetylxylan esterase, ferulic acid esterase, and *p*-coumaric acid esterase, which are necessary for hydrolyzing various substituted xylans (Figure 1(a)) (*46*), Endo-1,4-β-D-xylanases (EC 3.2.1.8) randomly cleave the xylan backbone, β-D-xylosidases (EC 3.2.1.37) cleave xylose monomers from the non-reducing end of xylo-oligosaccharides and xylobiose (Figure 1(b)). The side groups are catalyzed by α-L-arabinofuranosidases, α-D-glucuronidases, acetylxylan esterases, ferulic acid esterases and *p*-coumaric acid esterases (*56*).

The most important xylanolytic enzymes are the endo-β-1,4-xylanase, which hydrolyzes the insoluble xylan backbone into shorter, soluble xylo-oligosaccharides. At the beginning, the main hydrolysis products are β-D-xylopyranosyl oligomers, while at a later stage, small molecules such as mono-, di- and trisaccharides of β-D-xylopyranosyl may be produced (*58*). β-Xylosidase hydrolyzes the soluble xylo-oligosaccharides and xylobiose from the nonreducing end liberating xylose. β-Xylosidase plays an important role in xylan degradation by relieving the end product inhibition of endoxylanases (*59*). β-Xylosidase is also effective in transglycosylation reactions wherein monosaccharide units or alcohols are attached to or cleaved from xylose units. In the last decade, the interest in xylan-degrading enzymes has greatly increased due to their potential biotechnological applications (*56*). The α-L-AFases (α-L-arabinofuranoside arabinofuranohydrolases, EC 3.2.1.55) are the enzymes involved in the hydrolysis of L-arabinose linkages. The α-L-AFases specifically catalyze the hydrolysis of terminal nonreducing-α-L-1,2-, α-L-1,3- and α-L-1,5-arabinofuranosyl residues from different oligosaccharides and polysaccharides (*60–62*). The α-glucuronidases cleave 4-*O*-methyl-α-D-glucuronic acid (MeGA) from xylose residues when the MeGA is attached to the terminal, nonreducing end of xylo-oligosaccharides (*54*, *63*, *64*). The acetyl groups affect the capacity of other xylanolytic enzymes such as xylanases and α-glucuronidases to bind and hydrolyze backbone or side chain linkages (*65–67*). Hemicellulolytic esterases include acetylxylan esterase that hydrolyzes the acetyl substitutions on xylose moieties, ferulic/*p*-coumaric acid, which hydrolyze the ester bond linked either to lignin or to other ferulic/coumaric acid in xylans. Many xylanases do not cleave glycosidic bonds between xylose units that are substituted. The side chains must be cleaved before the xylan backbone can be completely hydrolyzed (*68*). These enzymes are produced by fungi, bacteria, yeast, marine algae, protozoans, snails, insect, seed, etc (*59*). The complete xylan-degrading enzyme systems have also been found. It is quite widespread among fungi (*59*, *69*), actinomycetes (*70*) and bacteria, and some of the most important xylanolytic enzyme producers including the *Aspergilli*, *Trichodermi*, *Streptomycetes*, *Phanerochaetes*, *Chytridiomycetes*, *Ruminococci*, *Fibrobacteres*, *Clostridia* and *Bacilli* (*55*, *71*, *72*) .

Ethanol

An important factor preventing industrial utilization of lignocelluloses for bioethanol production is the lack of microorganisms capable of efficiently ferment (with a high yield and a high rate) all sugars (both pentoses and hexoses) released during pretreatment and hydrolysis (*73*). The sugar mixture of hemicelluloses may contain any combination of xylose, arabinose, glucose, galactose, mannose, fucose and rhamnose depending on the source (*46*). This means that all types of sugars in cellulose and hemicelluloses must be converted into ethanol, and that microorganisms must be obtained that efficiently perform this conversion under industrial conditions. The pentose sugars, xylose and arabinose, which are not fermented to ethanol by the most commonly used industrial fermentation microorganisms, the yeast *Saccharomyces cervisiae*. Therefore, most research efforts have been devoted to the development of co-fermentation microorganisms.

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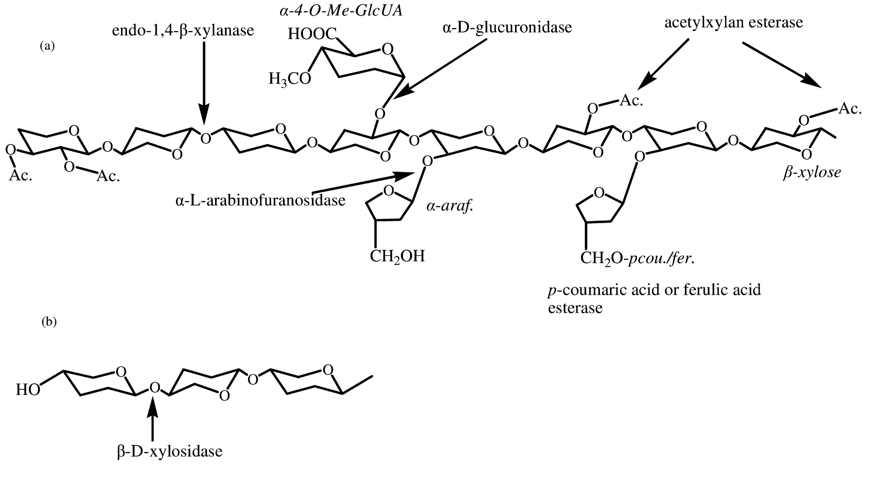
(a)
endo-1,4-β-xylanase
α-4-O-Me-GlcUA
HOOC
O
H3CO
α-D-glucuronidase
acetylxylan esterase
O
Ac.
O
Ac.
O
Ac.
α-L-arabinofuranosidase
α-araf.
CH2OH
CH2O-pcou./fer.
p-coumaric acid or ferulic acid esterase
β-xylose
(b)
HO
O
β-D-xylosidase

Figure 1. (a) Structure of xylan and the sites of its attack by xylanolytic enzymes. The backbone of the substrate is composed of 1,4-β-linked xylose residues. Ac., Acetyl group; α-araf., α-arabinofuranose; α-4-O-Me-GlcUA, α-4-O-methylglucuronic acid; pcou., p-coumaric acid; fer., ferulic acid. (b) Hydrolysis of xylo-oligosaccharide by β-xylosidase (57).

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Table 2. Pros and cons of various natural microorganisms with regard to industrial ethanol production (*36*, *86*)

Organism	*Natural sugar utilization pathways*					*Major products*		*Tolerance*			*O_2 needed*	*pH range*
	Glu	*Man*	*Gal*	*Xyl*	*Ara*	*EtOH*	*Others*	*Alcohol*	*Acids*	*Hydrolysate*		
Anaerobic bacteria	+	+	+	+	+	+	+	–	–	–	–	Neutral
E. coli	+	+	+	+	+	–	+	–	–	–	–	Neutral
Z. mobilis	+	–	–	–	–	+	–	+	–	–	–	Neutral
S. cerevisiae	+	+	+	–	–	+	–	++	++	++	–	Acidic
P. stipitis	+	+	+	+	+	+	–	–	–	–	+	Acidic
Filamentous fungi	+	+	+	+	+	+	–	++	++	++	–	Acidic

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Many different microorganisms have been used to produce ethanol, including bacteria, yeast, and filamentous fungi. In the processes of ethanol production from lignocellulosic materials, each group of microorganisms has its advantages and disadvantages. In Table 2, the substrate and product ranges of the most relevant microorganisms for ethanol production from hemicelluloses are summarized (*36*).

Anaerobic bacteria can ferment all lignocellulose-derived sugars, including their oligomers and polymers, to ethanol, other solvents, and acids (*74*). Anaerobic bacteria ferment pentose, but are inhabited already at low sugar and ethanol concentrations. In addition, ethanol fermentation occurs with considerable by-product formation, which reduces the ethanol yield (*75*). Furthermore, their optimal pH around 6–7 makes bacterial fermentation susceptible to infection and their low tolerance to lignocellulose-derived inhibitors requires a detoxification step to be included in the fermentation process (*76*).

Escherichia coli is the most efficient microorganism used both in industry and in the laboratory for protein expression, genetic studies, and molecular biology. It is able to ferment a wide spectrum of sugars, which include xylose and arabinose. *E. coli* dose not require complex growth factors, and it is one of the best characterized model organisms (*77*). However, there are several drawbacks: the narrow and neutral pH value at 6-8, low tolerance to lignocellulose-derived inhibitors, low ethanol tolerance, mixed-product (ethanol, acetic acid, lactic acid and others), and low ethanol yield (*78*).

In contrast to other bacteria, *Zymomonas mobilis* (Table 2) produces ethanol with stoichiometric yields and displays high specific ethanol productivity (*79–81*). Balat et al. (*82*, *83*) reported that *Z. mobilis* is able to achieve 97% theoretical ethanol yield, which is five times higher than *Saccharomyces cereisiae*. The remarkable ethanol yield obtained by *Z. mobilis* is a consequence of anaerobic D-glucose utilization using the Entner-Doudoroff (ED) pathway. The ED pathway yields only 1 ATP per molecule of glucose in contrast to 2 ATP per molecule of glucose via the Embden-Meyerhoff-Parnas (EMP), the common glycolytic pathway, which is present in both *E. coli* and *S. cerevisiae*. Therefore, more carbon is available for the production of ethanol (*79*). However, *Z. mobilis* has a narrow substrate range, which is limited to only glucose in the production of ethanol from lignocellulosic materials.

The best known microorganisms for ethanol production from hexoses are the yeast *Sacchamyces cerevisiae* offering high ethanol yields (90-97% of the theoretical) and high ethanol tolerance up to ca. 10% (w/v) in fermentation medium (*84*). On the basis of the initial glucose concentration, 99% ethanol can be obtained (*85*). *S. cerevisiae* has traditionally been used in large-scale ethanol fermentation of sugar- and starch-scale material and it is able to adapt to the industrial situation (*86*). It has a relatively good tolerance to lignocellulose-derived inhibitors and high osmotic pressure (*87*, *88*). Main disadvantage of the native strains of *S. cerevisiae* is its inability to metabolize and ferment the pentose sugars (xylose and arabinose) to ethanol.

Other microorganisms such as enteric bacteria and the yeasts *Pichia stipitis*, *Candida shehatae*, and *Pachysolen tannophilus* can be able to metabolize the main hemicelluloses sugar monomers and efficiently ferments xylose to ethanol (*89*, *90*). They are characterized by low ethanol yields and their tendency to re-assimilate the produced ethanol (*91*). *P. stipitis* is also described as being able to produc hemicellulolytic enzymes (*92*, *93*). Some species of aerobic filamentous fungi can tolerate industrial substrates well and ferment pentose sugars to ethanol, in addition to acids and hydrogen (*94–96*). Several filamentous fungi are the most efficient in hemicelluloses degradation, being the source of most commercial enzymes used in the production of lignocellulosic ethanol. However, filamentous fungi produce ethanol at low rates, and has limited tolerance to this alcohol (*97*).

In order to meet the requirements of industrial lignocelluloses fermentation, several microorganisms have been genetically engineered to overproduce ethanol from mixed sugar substrates by using two different approaches (*46*): 1) divert carbon flow from native fermentation products to ethanol in efficient mixed sugar utilizers such as *Escherichia*, *Erwinia*, and *Klebsiella*, and 2) introduce the pentose utilizing capability into efficient ethanol producers such as *Saccharomyces* and *Zymomobilis* (*98–101*). Pentose-fermenting *E. coli* (*102*) and *Klebsiella oxytoca* (*103*) have been generated by introducing ethanologenic genes from *Z. mobilis*. At the same time, the first xylose-fermenting *S. cerevisiae* strain was generated through the introduction of genes for xylosemetabolizing enzymes from *P. stipitis* (*104*). Zhang et al. (*101*) reported four genes from *E. coli* [xylA (xylose isomerase), xylB (xylulokinase), tal (transaldolase) and tktA (transketolase)] inserted *Z. mobilis*, and grew on xylose as the sole carbon source, and obtained 86% of the theoretical yield.

Xylitol

Xylitol is a five-carbon sugar polyol, which has been attracting the interest of food, odontological, and pharmaceutical industries. It is a compound with a sweetness similar to that of sucrose, is non-cariogenic, tolerated by diabetics, recommended for obese people. Because of its negative heat of dissolution, it can be also used as a part of the coating of pharmaceutical products (*105*, *106*). Although xylitol occurs in many fruit and vegetables, it would be very uneconomical to extract from such resources due to their high cost and relatively low xylitol content (*107*). Commercial production of xylitol is based on the nickel-catalyzed hydrogenation of xylose, which is extracted and purified from hemicellulosic hydrolysates. The critical step in this chemical process is the purification of the xylose from the acid hydrolysate. As a result, the high production costs limit the potential uses of xylitol, making it ten times more expensive than the process of sucrose to sorbitol. These reasons have stimulated the search for alternative method to produce xylitol. Today, one of most attractively alternative procedures is microbial, the use of xylose-fermenting microorganisms, such as the yeast *Candida guilliermondii* (anamorph form of *Pichia guilliermondii*), which directly converts xylose into xylitol, eliminating the need for xylose purification and the use of nickel as catalyst (*106*, *108*).

Xylose-to-xylitol bioconversion can be carried out by bacteria, filamentous fungi, and yeasts (*109*). Xylitol has been reported to be produced by bacteria such as soil isolate *Corynebacteriurn* sp. no. 208 (*110*), *Enterobacter liquefaciens* (*111*, *112*), and *Mycobacterium smegmatis* (*113*). Rangaswamy (*114*) reported 17 cultures belonging to three genera of facultative bacteria (*Serratia*, *Cellulomonas*,

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and *Corynebacterium*) were screened for the production of xylitol. A chromogenic assay of both solid and liquid cultures showed that ten of the 17 bacteria screened could grow on D-xylose and produce detectable quantities of xylitol during 24–96 h of fermentation. However, due to the relatively small quantities of formed xylitol, at present, xylitol-producing bacteria do not attract interests of researchers. There are few studies regarding xylitol production in filamentous fungi (*115–119*). Sampaio et al. (*119*) reported that eleven filamentous fungi were screened for xylitol production in batch culture, but the production was generally low under the growth conditions used in the Study. *Penicillium crustosum* presented the highest production, 0.52 g L^{-1} from 11.50 g L^{-1} of D-xylose, representing consumption of 76% of the original D-xylose. In general, among microorganisms, the yeasts are considered to be the best-known xylitol producers, especially species of genus *Candida* and *Debaryomyces* (*107*, *120–125*), such as *C. guilliermondii*, *C. pelliculosu*, *C. parapsilosis*, *C. tropicalis*, *C. pseudotropicalis*, *C. units*, and *Debaryomyces hansenii*. Other yeast genera investigated for xylitol production from xylose include *Saccharomyces, Debaryomyces, Pichia, Hansenula, Torulopsis, Kloeckera, Trichosporon, Cryptococcus, Rhodotorula, Monilia, Kluyveromyces, Pachysolen, Ambrosiozyma,* and *Torula* (*44*, *126*).

Some yeast carries out the conversion of xylose into D-xylulose through a two-step reduction and oxidation. In the first, in the presence of NADH (Nicotinamide Adenine Denucleotide Reduced Form) and/or NADPH (Nicotinamide Adenine Denucleotide Phosphate Reduced Form) xylose reductase (aldose reductase EC 1.1.1.21), xylose is reduced to the intermediate xylitol (*127*). In a subsequent reaction, xylitol is oxidized to xylulose by either NAD(P+)-dependent xylitol dehydrogenase (EC 1.1.1.9) and xylulose is then phosphorylated to xylulose-5-phosphate, which can be converted into pyruvate through a connection between pentose phosphate (PPP) and glycolytic pathways (*128–131*). The yeast *Debaryomyces hansenii* is able to grow in high saline media and has attracted some attention as a cell factory, especially because of its ability to produce polyols (*132*, *133*). Xylitol formation in this yeast is hindered by anaerobic conditions, owing to a negligible NADH-dependent xylose reductase activity; in fact, NADH accumulated in the cytosol can be only partially regenerated thus leading to ethanol production (*134*, *135*). Under excess aerobiosis, oxygen can reoxidize NADH by the respiratory chain, stimulating growth and affecting xylitol production. Therefore, xylitol formation can only be ensured by semi-aerobic conditions, under which oxygen is just sufficient to regenerate NADH, while NADPH produced by the pentose-phosphate pathway is almost entirely addressed to xylitol formation (*121*, *136*).

Studying the production of xylitol is of particular interest as a prerequisite for raising the xylitol production and yield by optimizing xylitol production conditions. Studies on the biotechnological production of xylitol from xylose-rich lignocellulosic materials have demonstrated that this process can be influenced by the type of hemicellulosic hydrolysate as well as by the fermentation conditions employed in the process, such as temperature, pH, oxygen availability, nutrients concentration in the fermentation medium, xylose concentration, and the presence of compounds toxic to the microbial metabolism (*137*). Rao et al. (*120*) evaluated the ability of *C. tropicalis* for xylitol production from corn fiber and sugarcane bagasse hydrolysates. Owing to the presence of inhibitors in these hydrolysates, xylose uptake and xylitol production were very low, even after hydrolysate neutralization and treatments with activated charcoal and ion exchange resins. To improve yeast growth and xylitol production with these hydrolysates, which contain inhibitors, the cells were adapted by sub-culturing in the hydrolysate containing medium for 25 cycles. After adaptation the organism produced, more xylitol 0.58 g g^{-1} and 0.65 g g^{-1} of xylose were obtained from corn fiber hydrolysate and sugarcane bagasse hydrolysate, respectively.

Continuous and fed-batch culture techniques often provide better yields and productivities in the production of microbial metabolites than batch cultures (*138*). Continuous fermentations often offer higher productivities of metabolites only at low dilution rates, which need long residence times. For practical reasons, some continuous operations have been replaced by fed-batch processes (*139*). Batch fermentation has been exploited extensively for the production of xylitol. In order to maintain the growth rate and substrate concentration at suitable levels during the whole period of cultivation, Vandeska et al. (*140*) investigated xylitol production by *C. boidinii* NRRL Y-17213 in fed-batch fermentations. He found at the first phase, under the highest initial xylose concentration (100 g L^{-1}) and the lowest level of aeration, the highest yield of xylitol (75% of theoretical) and the highest productivity (approximately 35% higher) were obtained.

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2,3-Butanediol

2,3-Butanediol (2,3-BD) is also known as 2,3-butylene glycol, dimethylene glycol, dimethylethylene glycol and the IUPAC name is butane-2,3-diol. It is a potential bulk chemical that can be produced by a variety of microorganisms through microbial fermentation (*141*). 2,3-BD is a valuable chemical feedstock because of its application as a solvent, liquid fuel, and as a precursor of many synthetic polymers and resins (*46*). One of well known applications is the formation of methyl ketone, by dehydration, which can be used as a liquid fuel additive. The heating value of 2,3-BD (27198 J g^{-1}) compares favorably with other liquid fuels, e.g. methanol (22081 J g^{-1}) and ethanol (29055 J g^{-1}) (*142*). Equimolar mixture of ethanol and 2,3-BD can provide a combined heating value of 27660 J g^{-1}, so the presence of ethanol does not affect the usefulness of 2,3-BD in this application (*143*). Methyl ketone can be used as a solvent for resin and laquers (*144*). 2,3-BD is also to be converted to 1,3-butadiene, which is a substance used in the production of synthetic rubber. Owing to its low freezing point of -60 °C, 2,3-BD is used as an antifreeze (*145*). Esterification of butanediol forms precursors of poly urethane foams for use in drugs, cosmetic products, lotions, ointments, and antiperspirants, etc. The dehydrogenation of 2,3-BD produces diacetyl, which is a valuable flavoring agent and bacteriostatic food additive for the food industry (*146*). Butanediol has also been shown to have potential application in the printing inks, perfumes, fumigants, spandex, moistening and softening agents, plasticizers and carrier for pharmaceuticals (*141*).

As early as in 1906, Harden and Walpole investigated the microbial production of 2,3-butanediol employing *Aerobacter aerogenes* (*Klebsiella*

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pneumoniae) and this was followed by Donker and Kluyver using *Bacillus polymyxa* (*147*). Fulmer *et al.* (*148*) proposed the industrial production of the diol for the first time. Attention was again focused on diol fermentation during wartime due to the anticipated shortage of 1,3-butadiene (*141*). 2,3-BD can occur in three stereoisomers, the *dextro*-, *levo*- and *meso*-forms. Depending on the microorganism and the cultivation conditions, the ratio of 2,3-BD stereoisomers can vary dramatically. *Bacillus polymyxa* produce *levo*-form of 2,3-BD, whereas *Klebsiella pneumoniae* (*Aerobacter aerogenes*) produce meso-form and also some of the *levo*-form. *Bacillus subtilis*, *Seratia marcescens* and *Aerobacter hydrophia* produce mixtures of different forms (*149*). *Klebsiella pneumoniae* is the organism of choice owing to its ability of utilizing all the major sugars (hexoses, pentoses, certain disaccharides) and uronic acid derived from the hydrolysates of hemicellulosic and cellulosic materials (*143*, *150*, *151*). *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Enterobacter aerogenes* produce dextro- and meso-stereoisomer of 2,3-BD (*152*). In *Bacillus polymyxa*, the *levo*-form of 2,3-BD is produced as a major product, a purity of over 98% can be obtained from this wild-type strain (*153*, *154*). *K. pneumoniae*, *K. oxytoca*, and *B. polymyxa* are considered to be strong candidates for efficient production of 2,3-BD, and much efforts have been devoted to the optimization of growth conditions for maximal 2,3-BD productivity and yield (*154–158*).

2,3-BD fermentation is influenced by various factors: aeration, pH, temperature, and substrate (*141*, *152*). Oxygen supply is consider to be the most important factor for 2,3-BD fermentation by the native producer. 2,3-BD is produced under low O_2 supply, and, as such, is a classical example of anaerobic fermentation in terms of maintaining an internal redox balance with respect to the pyridine nucleotide pool during glycolysis and biosynthesis (*159*). It governs the distribution of fermentation end-products and controls the yield of 2,3-BD (*152*, *154*, *155*, *160*). Li et al. altered the mixed acid fermentation pathways of *E. coli* and studied the production of meso-2,3-butanediol under low oxygen conditions (*161*). In a 6-L fermentor supplied with only 3% dissolved oxygen (DO), the mutant harboring pEnBD converted glucose to 2,3-BD much faster than the control did. When DO supply was further lowered to 1% DO, the recombinant mutant grew much slower but produced 2,3-BD as a major fermentation metabolic product. In addition, the 2,3-BD yield showed an increase from 0.20 g BD g^{-1} glucose for the control to 0.43 g BD g^{-1} glucose for the mixed acid pathway deleted mutant grown in fermentors under 1% DO. Moes et al. (*162*) reported that the mentioned increase in acetoin production increased with the O_2 supply increasing. They found that the reaction acetoin↔2,3-BD was reversible, switching the DO level in this range would cause conversion of one product to another in a reversible manner. When acetoin was excreted, the dissolved DO level was above 100 ppb, and 2,3-BD production was prevailing, as DO was below100 ppb. The crucial DO level was 80-90 ppb when the product concentration ratio changed rapidly. The pH is a fundamental parameter in the regulation of bacterial metabolism and its influence is especially pronounced in the processes involving multiple end-products formation. As a general rule, organic acid is easily formed in the alkaline conditions (pH, 6.3-6.5), with a simultaneous decrease in the 2,3-BD yield, whereas under the acidic conditions, organic acid synthesis is reduced about 10-fold, and diol production increases 3-7 times (*141*). Grover et al. (*163*) reported that a correlation between butanediol, ethanol production and growth of *K. pneumoniae* cultured in wood hydrolysate at pH 6.0 and 30 °C . Volochet et al. (*164*) found the optimum pH value ranged from 5 to 6 for 2,3-BD production. Celinska and Grajek (*152*) reviewed the biotechnological production of 2,3-BD and found pH value of 5.5 was cited as the most frequently value reported in literature for the process. They tested pH value between 4.0 and 8.0. The conversion yield was nearly constant within a narrow range of pH 5.0-6.5, and it sharply decreased either at lower or at higher pH values. The strongest effect was observed under acidic conditions, which stemsed from the fact that the lower pH value usually inhibits the biomass growth as well as the bioprocess itself (*165*). The efficiencies of bioprocesses become strictly temperature-dependent owing to the strong dependence of enzymatic activity and cellular maintenance upon temperature (*141*). In general, the optimum temperature for the bacterial fermentation of butanediol is reported to be in the range of 30-35°C (*166*). A variety of carbohydrates can be used to produce 2,3-BD by microbial fermentation, and particularly from bacterial fermentation, utilizing hexose or pentose (*167*). For example, *Aerobacter aerogenes* can be used as a carbon source for conversion to 2,3-BD (*168*). In the most processes, the concentrations of carbohydrates are in the range of 5-10% (*141*). Yu and saddler (*143*, *150*) reported that *K. pneumoniaw* which grew on high initial sugar concentrations (up to 10%) in the presence of acetic acid can efficient utilize xylose to produce 2,3-BD.

In addition, as for the nitrogen sources in 2,3-BD fermentation, the previous works have investigated yeast extract or ammonium salts (*169*, *170*). Because of their relatively high cost, urea as a nitrogen source was used for replacing ammonium salts. On an equivalent nitrogen basis, urea is much cheaper than yeast extract and is typically half the cost of ammonium salt. Also, using urea as nitrogen source has additional benefits. Unlike ammonium salts, the metabolism of urea does not contribute to the acidification of the medium, thus reduces the amount of base required for pH control (*171*). Ji et al. (*157*) reported an industrial medium containing urea as a sole nitrogen source, low levels of corn steep liquor and mineral salts as nutrition factors to retain high 2,3-butanediol production through co-fermentation of glucose and xylose (2:1, wt/wt) by *Klebsiella oxytoca* was developed. Urea and corn steep liquor were identified as the most significant factors by the two-level Plackett–Burman design. Under the optimal medium, the yield of 2,3-butanediol plus acetoin relative to glucose and xylose was up to 0.428 g g^{-1}, which was 85.6% of theoretical value. The cheap nitrogen source and nutrition factors combining the co-fermentation process using glucose and xylose as the carbon source in the developed medium would be a potential solution to improve the economics of microbial 2,3-butanediol production.

Other Value-Added Chemicals

Besides ethanol, xylitol, and 2,3-BD, other industrially significant chemicals such as lactic, itaconic and ferulic acids, coumaric acid, xylonic acid, alactaric and galactonic acids, and single cell protein can be manufactured form hemicellulosic

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sugars. These chemicals have wide applications in the food, feed, pharmaceutical, and cosmetics industries (*44*, *172*).

Lactic acid and its derivatives represent an important category of compounds for industries producing food, chemical and pharmaceutical products. Lactic acid can be synthesized chemically, but the obtained products are the racemic mixtures of D- and L-isomers. Chirally pure lactic acid can be produced by microbial fermentation and is required for the manufacturing of the biodegradable polymer polylactic acid, which is an alternative to petrochemically derived plastics (*173*). Furthermore, the microbial production of lactic acid is of economic importance due to the prospect of using cheap and widely available feedstock materials such as lignocellulose (*174*). Maas et al. (*175*) studied the conversion of xylose to lactic acid by ten diVerent wild-type strains of *R. oryzae*. When cultures of *R. oryzae* CBS 112.07 were exposed to relatively high xylose concentrations (60–120 g L^{-1}), only $\pm$40 g L^{-1} xylose was converted to mainly lactic acid. They also demonstrated that the production of lactic acid by *R. oryzae* CBS 112.07 only occured under growing conditions (*176*). In order to achieve efficient homo D-lactic acid production from xylose, Yoshida et al. (*177*) introduced a plasmid carrying the xylAB operon from *Lactobacillus pentosus* (pCU-PXylAB) into several mutants of an L-lactate dehydrogenase gene (ldhL1)-deficient *L. plantarum* (DldhL1). The DldhL1 transformant assimilated xylose via the phosphoketolase pathway that produced an equimolar amount of lactic acid and acetic acid as final products. The strain harboring pCU-PXylAB produced predominantly lactic acid from xylose via the pentose phosphate pathway and successful efficient homo D-lactic acid production was achieved and 41.2 g L^{-1} of lactic acid was produced from xylose with a high yield (0.89 g g^{-1} of consumed sugar) and an optical purity of D-lactic acid of 99.2 k.

Single-cell protein (SCP) refers to the dried cells of microorganisms such as yeasts, bacteria, fungi and microalgae, which grow in large-scale culture systems for use as protein sources in human food or animal feed. Not only proteins, but also free amino acids, lipids, carbohydrates, vitamins and minerals are often included in the single-cell protein term (*178*). Many microorganisms have been used to convert various substrates and industrial waste products into high valuble chemicals (*179*, *180*). Microorganism *C. blankii* UOVS-64.2 was employed for SCP production from hemicellulose hydrolysates, and was increased by intraspecific protoplast fusion of auxotrophic mutants produced by UV irradiation followed by nystatin enrichment (*181*).

Chemicals from Hemicelluloses via Chemical Transformations

Hemicelluloses are carbohydrate-based polymers that can be broken down to low molecular weight sugars by hydrolysis using an acid catalyst. As shown in Figure 2, the acid-catalysed decomposition of the C6-sugar fragments (e.g., glucose) leads to 5-hydroxymethyl-2-furaldehyde as the intermediate product, and subsequently rehydrated to give levulinic and formic acids as the final products. Hydrolysis of the C5 sugars of hemicelluloses may lead to furfural. In addition, other constituents in the hemicelluloses matrix may produce side products like acetic acid and galacturonic acid (*181*). The simplified reaction scheme is given

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in Figure 2. In this section, we will discuss the main chemicals coming from the sugar in hemicelluloses by chemical transformation.

Furfural

Furfural is produced from agricultural waste biomass, mainly containing pentosans (mainly xylose), such as corn cobs, sugar cane bagasse, rice and oat hulls etc (*182*). The other resources are paper-pulp residue, bamboo, kenaf, grain hulls, wheat and rice straw, nut shells, cottonseed and wood (soft and hardwood). Furfural is a clear, colorless motile liquid with a characteristic 'almond-benzaldehyde' odor. The molecular formula is $C_5H_4O_2$. Its synonyms are: 2-furancarboxaldehyde, furaldehyde, 2-furanaldehyde, 2-furfuraldehyde, fural, and furfurol (*183*).

The worldwide furfural market reaches around 300,000 metric/year, and its price (250 euros t^{-1}) are in the range of basic petrochemicals, such as benzene and toluene (225-250 euros t^{-1}) (*10*, *184*). It is directly used as a solvent in petroleum refining and in the production of phenolic resins and is the basis for synthesizing a family of derived solvents, including furfuryl alcohol, furfurylic alcohol, and tetrahydrofuran (THF) (*185*). Feedstocks for furfural production are those with high pentosan content; potential furfural yields for typical feedstocks, expressed in kg of furfural per metric ton of dry biomass, are 220 for corncobs, 170 for bagasse, 165 for cornstalks, 160 for sunflower hulls, 120 for rice hulls and around 150-170 for hardwoods (*184*).

Furfural is commercially synthesized from agricultural raw materials in a process which involves acid hydrolysis of the pentosans into pentoses and successive cyclodehydration of the latter to form furfural (Figure 3) (*186*). The first step is acid hydrolysis of the pentosans into pentoses. Among the treatments of hydrolysis, dilute acid hydrolysis appears to be in the best position from the economic viewpoint (*187*). Dilute acids lead to a limited hydrolysis called prehydrolysis. This consists in the hydrolysis of the hemicellulosic fraction, remaining the cellulose and lignin fractions almost unaltered (*188*). Sulphuric (*189*), hydrochloric (*190*, *191*), hydrofluoric (*192*) or acetic acids (*193*) are commonly employed as catalysts. Recently, phosphoric acid has been proposed for the hydrolysis of sugar cane bagasse (*194*). The hydrolysis reaction in dilute-acid medium is very complex, mainly because the substrate is in a solid phase and the catalyst in a liquid phase. The mechanism of the hydrolysis reaction includes (*195*): 1) diffusion of protons through the wet lignocellulosic matrix; 2) protonation of the oxygen of a heterocyclic ether bond between the sugar monomers; 3) breaking of the ether bond; 4) generation of a carbocation as intermediate; 5) solvation of the carbocation with water; 6) regeneration of the proton with cogeneration of the sugar monomer, oligomer or polymer depending on the position of the ether bond; 7) diffusion of the reaction products in the liquid phase if it is permit for their form and size (some big oligomers cannot cross the matrix); 8) restarting in the second step. Therefore, the modeling of the hydrolysis of a polysaccharide is very complicate and empirical models are commonly used (*188*). The second step of dehydration is comparatively slower than that of mineral acid. The rate of the first step of the furfural production

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process, hydrolysis reaction, on average is about 50 times higher than that for the second step, the dehydration reaction (*196*). Hence, the furfural formation process limiting stage is the dehydration reaction. For commercial scale biomass conversion, the most important target is a maximal generation of pentose and minimal loss of cellulose. The formation of furfural from pentose is represented in Figure 3, xylose in the presence of acid catalysts, mainly sulfuric acid, undergos a triple dehydration, and then finally forms furfural (*197*).

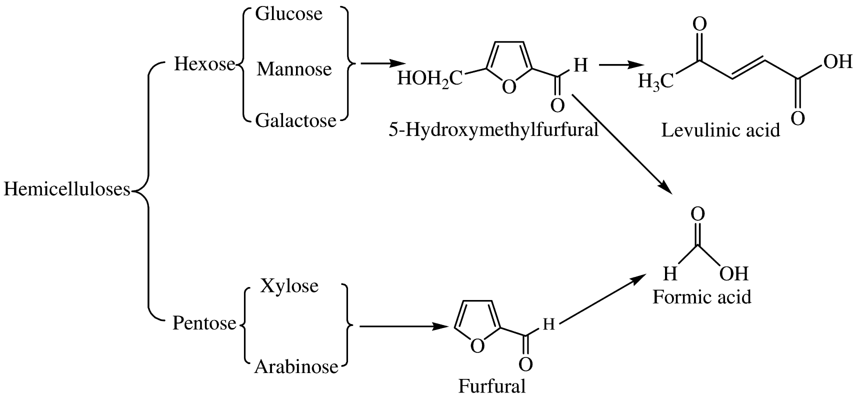
Hemicelluloses
Hexose
Glucose
Mannose
Galactose
Pentose
Xylose
Arabinose
5-Hydroxymethylfurfural
Levulinic acid
Formic acid
Furfural

Figure 2. Chemicals obtained by dehydration of monosaccharides.

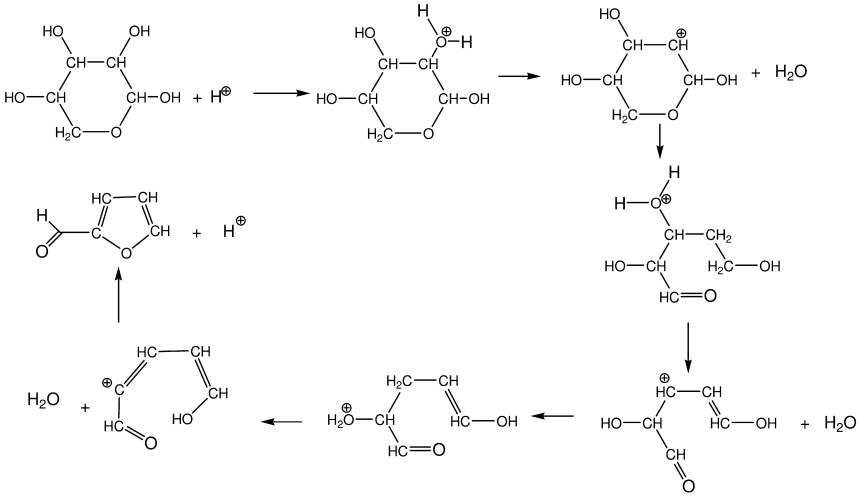

Figure 3. The mechanism for the dehydration of pentosan to furfural (197).

In 1922, the first commercial process for production of furfural was developed by Quaker Oat Company from oat hulls using by sulfuric acid catalyst (*198*). Furfural is at present obtained through dehydration of pentoses, especially xylose, or hemicelluloses, at high temperatures (200-250 °C) and in the presence of mineral acids as catalysts, mainly sulfuric acid (*199*). Under these conditions, the selectivities in furfural do not exceed 70%. In order to enhance the efficiency of furfural recovery, the use of solvent in liquid-liquid extraction is recommended. Steam distillation is often used to recover furfural from the reaction mixture. The diffusion of furfural through the liquid surrounding the solid substrate into the stripping steam is slow, and produces the high-energy consumption. Thus this is inefficient (*200–202*). Alternatively, the supercritical fluid can quickly separate the highly reactive product from a reactor to suppress the side reactions (*203–206*). Sako et al. (*207*) investigated the effect of the extraction of furfural by supercritical CO_2 on the selectivity, and the yields of furfural exceed 80%. Sangarunlert et al. (*208*) investigated the optimum condition for furfural production from rice husk by hydrolysis accompanying supercritical CO_2 extraction. The two-level fractional factorial design method was used to investigate the production process carried out with respect to furfural yield. The process variables are temperature range of 373-453 K, pressure 9.1-18.2 MPa, CO_2 flow rate 8.3×10^{-5}-1.7×10^{-4} kg s^{-1} (5-10 g min^{-1}), sulfuric acid concentration 1 to 7 (%wt) and ratio of liquid to solid (L/S) 5:1 to 15:1 (vol/wt). The two-stage process (pre-hydrolysis and dehydration) in furfural production can improve furfural yield further to be around 90% of theoretical maximum. With the aim of finding potential industrial applications, attempts have been made to develop heterogeneous catalytic processes for the transformation of pentosans/pentoses into furfural that offer environmental as well as economic benefits (*209*). Moreau et al. (*210*, *211*) reported if conversion is kept at about 30%, the high selectivity (90–95%) was obtained under the interesting catalysts, microporous materials such as zeolites (faujasites and mordenites). The other heterogeneous catalysts including micro-mesoporous sulfonic acid catalysts and heteropolyacids also have been investigated (*212*, *213*). Unfortunately, the catalytic performances achieved up to now have been unsatisfactory for industrial implementation.

There are numerous useful compounds derived from furfural. The most important compounds are illustrated in Figure 4. Furfural can be hydrogenated to furfuryl alcohol, methyl furan, tetrohydrofurfural alcohol, and methyltetrahydrofuran (MTHF). Furfuryl alcohol (2-furan methanol) is an important chemical in polymer industry (*214*, *215*), which can be mainly used for the production of thermostatic resins, liquid resins used for strengthening ceramics. Furfuryl alcohol can also be used in producing various synthetic fibers, rubber-resins and farm chemical (*216*, *217*). Furfural is also a useful compound that is used in the manufacture of pharmaceuticals, pesticides, fibers, etc.

5-Hydroxymethylfurfural

5-Hydroxymethylfurfural (HMF), the hexose dehydration product, is an important intermediate in the chemical transformation of carbohydrate from biomass (*43*). Several reviews have been reported concerning the chemistry of

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5-hydroxymethylfurfural (*218–220*). In the 19th century, 5-hydroxymethylfurfural was already studied. Düll and Kiermeyer (*221*, *222*) independently published a method of synthesis and chemical reactions of the compound, which they called "oxymethylfurfurol". The synthesis of HMF is based on the triple dehydration of hexoses. The hexoses (maily glucose, fructose and mannose), oligo- and polysaccharides as well as converted industrial wastes can be utilized as HMF material. As can be seen from Figure 5, besides HMF, various side-dehydration products, such as levulinic acid and humic acids, can be produced in the reaction of the acid catalyzed dehydration (*223*). The decomposition to levulinic acid and the polymerization to humic acids are the most important factors, which make the yield of HMF decrease (*219*). Antal et al. (*227*) found other side-reaction in the course of sugar decomposition, such as isomerisation, fragmentation and condensation. In addition, Van Dam and Corrier (*224*, *225*) found in the aqueous and non aqueous processes about 37 products were produced. Then Haworth and Jones (*226*) worked out the modern synthesis method of HMF and studied the mechanism of dehydration. They firstly reported the mechanism of the dehydration of fructose leading to HMF. In the subsequent research, Van Dam (*224*), Antal (*227*), and Kuster (*228*) demonstrated the dehydration of hexoses (especially fructose and glucose) could go through by two possible pathways (Figure 5). One pathway was based on acyclic compounds, and the other was on the basis of the transformation of ring systems.

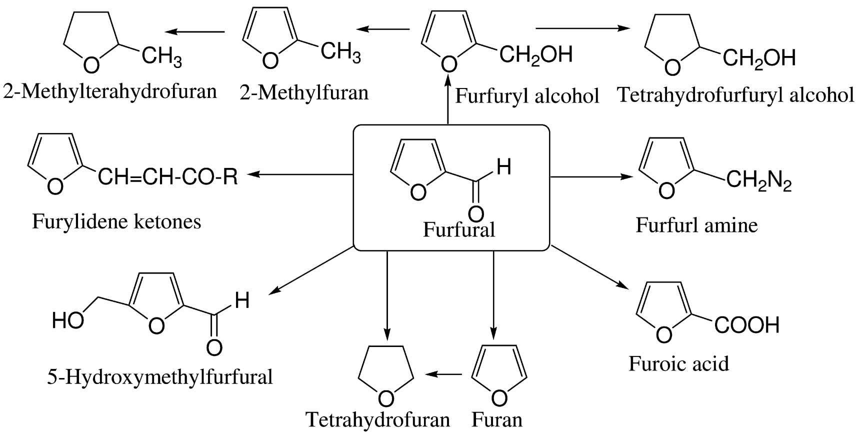
CH3
2-Methylterahydrofuran
CH3
2-Methylfuran
CH2OH
Furfuryl alcohol
CH2OH
Tetrahydrofurfuryl alcohol
CH=CH-CO-R
Furylidene ketones
H
O
Furfural
CH2N2
Furfurl amine
HO
H
O
5-Hydroxymethylfurfural
Tetrahydrofuran
Furan
COOH
Furoic acid

Figure 4. Primary derivative from furfural (10).

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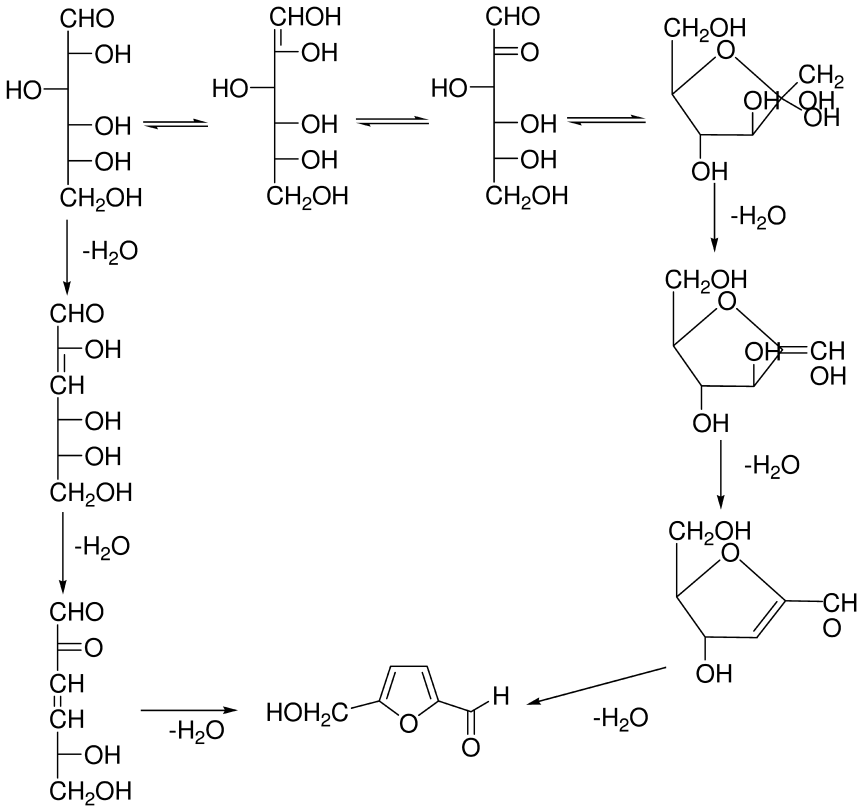

Figure 5. The mechanism of the hexoses dehydration (219).

The first preparation of HMF used sucrose as feedstock, and while the glucose part stayed largely uncovered, the molar yield of only 25% (50% based on the fructose part) were obtained (*226*, *229–231*). The best raw material for HMF manufacture is crystalline fructose, fructose rich syrups or inulin hydrolysate (*228*). However, glucose is still used in industry as a source of HMF because its cost is lower than that of fructose (*218*). The most convenient for the preparation of HMF is the acid-catalyzed dehydration of fructose (*10*). The dehydration of hexose is catalyzed by various catalysts. Cottier (*218*) divided catalysts into five groups: organic acids, inorganic acids, salts, Lewis acids, and ion-exchange resins as well as zeolites (Table 3) (*221*, *222*, *226*, *231–247*). The type of solvent and its influence on the efficiency of the dehydration is closely connected with temperature conditions. Cottier (*218*) divided methods into 5 groups depending on the type of solvent and the temperature of the process: 1) Aqueous processes carried out at temperatures below 200 °C; 2) Aqueous processes carried out at temperatures over 200 °C; 3) Processes in non-aqueous medium; 4) Processes in mixed solvents; 5) Processes without solvent and microwave processes.

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Table 3. Group of catalysts for dehydration of hexoses (*219*)

Organic acids	oxalic acid, levulinic acid, maleic acid, *p*-toluenesulfonic acid
Inorganic acids	phosphoric acid, sulfuric acid, hydrochloric acid, iodine or hydroiodic acid generated in situ
Salts	$(NH4)_2SO_4/SO_3$, pyridine/PO_{4-3}, pyridine/HCl, aluminum salts, Th and Zr ions, zirconium phosphate, Cr; Al, Ti, Ca, In ions, $ZrOCl_2$, $VO(SO_4)_2$, TiO_2, V, Zr, Cr, Ti porphyrins
Lewis acids	$ZnCl_2$, $AlCl_3$, BF_3
Others	ion-exchange resins; zeolites

As far as the reaction medium is concerned, water is the most frequently used solvent, due to its excellent substrate solution properties and low cost. However, under the above conditions rehydration of HMF to levulinic and formic acids easily occurred, strongly limiting the use of the aqueous medium, and the yields of HMF were low (30%) (*233*, *248*). Early work showed that HMF could be strongly reduced humins formation and be produced in high yields using high-boiling nonaqueous solvents, such as dimethylformamide (DMF), acetonitrile and dimethylsulfoxide (DMSO) (*249–251*). Mussau et al. (*251*) performed the reaction without a catalyst in DMSO. Nakamura et al. (*252*) obtained HMF in 80% yield using DMSO as a solvent and a strongly acidic ion-exchange resin as catalyst. The advantage of using nonaqueous solvents is that it prevents the formation of levulinic and humic acids. Unfortunately, the removal of these organic solvents and the purification of HMF may give rise to additional technical difficulties and to environmental problems. Moreover, with hydrosoluble raw materials, as the above carbohydrates, a large amount of organic solvents is required, due to their scarce solution capability, thus implying high substrate dilution and consequently reduced productivity (*253*). In order to improve the selectivity towards the target product, the use of mixed solvents (water-organic) in homogeneous aqueous solution has been also suggested a positive effect. Experiment performed in aqueous butanol, dioxane and poly (ethylene glycol) showed a decrease in the levulinic acid formation (*233*, *234*, *254–256*). Moreover, biphasic systems were also applied, the organic component behaving as extracting agent. When methyl isobutyl ketone (MIBK) was used as organic co-solvent, the extraction of HMF strongly inhibited its degradation to levulinic and formic acids. Chheda et al. (*257*) studied the production of HMF and furfural by dehydration of fructose, glucose and xylose using a biphasic reactor system, comprised of reactive aqueous phase modified with DMSO, combined with an organic extracting phase consisting of a 7:3 (w/w) MIBK-2-butanol mixture or dichloromethane (DCM). Experiments with the MIBK-2-butanol mixture were conducted at a temperature of 443 K using mineral acid catalysts (HCl, H_2SO4 and H_3PO4) at a pH from 1.0 to 2.0, whereas experiments with DCM as the extracting solvent were conducted at 413 K and did not require the use of an acid catalyst. The modifiable nature of the biphasic system allowed us to identify preferred DMSO and pH levels for each sugar to maximize the HMF selectivity at high sugar conversions, leading to selectivities of 89%, 91%, and 53% for dehydration of fructose, xylose, and glucose, respectively.

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In recent years, the effective conversion of fructose into HMF in water, organic solvents, and multiphase systems has been reported. Dumesic and co-workers (*258*, *259*) studied acid-catalyzed fructose dehydration in a two-phase reactor system at 180 °C, and achieved a maximum 5-HMF selectivity of 85% with 89% fructose conversion. Binder et al. (*260*) developed a process that converted fructose to 5-HMF in *N,N*-dimethylacetamide in the presence of KI and H_2SO_4 at 100 °C for 5 h reaction time, and achieved a 5-HMF yield of 92%. Ionic liquids have been studied for the production of HMF, and obtained some good results. Preliminary studies showed that, when the reaction was performed using 1-butyl-3-methylimidazolium tetrafluoroborate as solvent and Amberlyst-15 as catalyst, up to 50% yield of HMF was obtained within around 3 h at 80 °C. However, using DMSO as co-solvent, yields of HMF close to 80% were achieved within 24 h (*213*, *261*). Zhao et al. (*262*) achieved a 5-HMF yield of 83% in an ionic liquid solvent (1-ethyl-3-methylimidazolium chloride, [EMIM][Cl]) with $CrCl_2$ as catalyst at a temperature of 80 °C for a reaction time of 3 h. Yong et al. (*263*) studied the production of 5-HMF from fructose in 1-butyl-3-methyl imidazolium chloride ([BMIM][Cl]) using $CrCl_2$ as catalyst, and 5-HMF yields of 96% were achieved at 100 °C in 6 h reaction time. Qin et al. (*170*) realized the conversion of fructose into HMF at room temperature and in quantitative yields (90.3%). They predissolved the fructose into the [BMIM][Cl], and reaction mixture was kept at room temperature (25 °C). Binder and Raines (*260*) reported that *N,N*-dimethylacetamide (DMA) containing lithium chloride (LiCl) was a privileged solvent that enables the synthesis of the renewable platform chemical HMF in a single step and unprecedented yield from untreated lignocellulosic biomass, as well as from purified cellulose, glucose, and fructose. They also showed that ionic liquid systems could enable the conversion of glucose or cellulose to high-yield HMF, but ionic liquids themselves were so expensive that they were difficult to apply commercially (*264*, *265*).

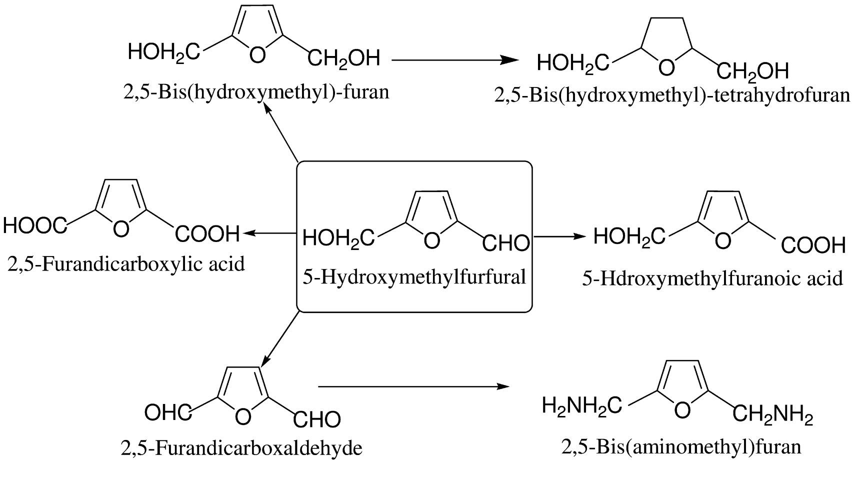
HOH2C CH2OH
2,5-Bis(hydroxymethyl)-furan
HOH2C CH2OH
2,5-Bis(hydroxymethyl)-tetrahydrofuran
HOOC COOH
2,5-Furandicarboxylic acid
HOH2C CHO
5-Hydroxymethylfurfural
HOH2C COOH
5-Hdroxymethylfuranoic acid
OHC CHO
2,5-Furandicarboxaldehyde
H2NH2C CH2NH2
2,5-Bis(aminomethyl)furan

Figure 6. Primary derivative from 5-hydroxymethylfurfural (10).

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Among many chemicals derived from biomass, HMF is considered to have the potential to be a sustainable substitute for petroleum-based building blocks (*266*). HMF is a good starting material for products including resins, adhesives, coatings, composites, foams, sealants, binders, curatives, solvents, monomers, and pharmaceuticals. The most versatile intermediate chemicals of high industrial potential that can be generated from HMF in simple large-scale transformations are 5-hydroxymethylfuranoic acid, 2,5-furandicarboxylic acid (FDCA), 2,5-bis(hydroxymethyl) furan, and 2,5-furandicarboxaldehyde (DFF) (Figure 6) (*10*). HMF can be converted to FDCA by selective oxidation, which can replace terephthalic, isophthalic, and adipic acids in the manufacture of polyamides, polyesters, and polyurethanes (*209*, *267*). HMF and FDCA are so-called “sleeping giants” and hold a key position in biomass-derived intermediates. Furandicarboxaldehyde, or 2,5-diformylfuran (DFF), is a versatile compound, and there are numerous reports describing various useful applications as a monomer (*267*, *268*), as a starting material for the synthesis of pharmaceuticals and antifungal agents (*269–271*), as a constituent in photography (*272*), as a component for foundry sand binders (*273*), etc. 2,5-Furandicarboxylic acid 2,5-Bis(hydroxymethyl) furan (BHMF) is a valuable product in the furan family, and is useful as an intermediate in the synthesis of drugs, crown ethers, and polymers (*274–277*). HMF has been used for the production of special resins, and lots of other polymerizable furanic monomers with promising properties have been prepared (*278*). In addition, HMF can serve as a precursor in the synthesis of liquid alkanes to be used, for example, in diesel fuel (*279*).

Levulinic Acid

Levulinic acid (LA), also known as 4-oxopentanoic acid or γ-ketovaleric acid, is a C5-chemical with a ketone and a carboxylic group. The presence of both groups results in interesting reactivity patterns (*280*). LA is readily soluble in water, ethanol, diethyl ether, acetone and many other organic solvents. The dissociation constant (pK_a) of LA is 4.59, which is comparable with low molecular weight aliphatic carboxylic acids (*281*). LA is a short chain fatty acid having a ketone carbonyl group and an acidic carboxyl group and is a very versatile building block for the synthesis of (bulk) chemicals for applications, which can be used as textile dye, antifreeze, animal feed, coating material, solvent, food flavoring agents, pharmaceutical compounds and resin (*282*). Several reviews have been published describing the properties and potential industrial applications of levulinic acid and its derivatives (*283–286*).

In the 1840s, Mulder, who prepared LA by heating sucrose with mineral acids at high temperature, firstly reported the preparation of LA, which prepared LA by heating sucrose with mineral acids at high temperature (*287*). The controlled degradation of hexose (C6-sugars) by acids is still the most widely used approach to prepare LA from lignocellulosic biomass. LA can be produced by heating hexose, or any carbohydrate containing hexose with a dilute mineral acid for an extended time (*288*). The acid catalyzed degradation of hexoses into LA has been extensively studied (*224*, *226*, *289*, *290*). Horvat et al. (*291*) reported the mechanism of production of LA on the basis of analysis of ^{13}C NMR of the reaction mixture. The available information implies that hexose sugars initially dehydrate to form the intermediate product HMF, which is subsequently hydrated to give the final product LA. The mechanism for the conversion of hexose sugars (glucose, mannose or fructose) to HMF has been introduced in Figure 7. The conversion of HMF into LA is the result of water addition to the C_2 -C_3 bond of the furan ring to give the final products LA and formic acid (Figure 7). Other starting materials and reagents have also been used for production of LA, such as the acid hydrolysis of furfuryl alcohol (*292*, *293*), the hydrolysis of acetyl succinate ester (*294*), and the oxidation of ketones (*295–297*). Using the Pd-catalysed carbonylation of ketones (*298*), the alkylation of nitroalkanes (*299*), and 4-(dephenylmethysilyl)butyrolactone with MeMgI (*300*) can also obtain the high yield of LA. However, these methods cannot compete on an industrial scale with the simple and inexpensive procedures used in the preparation of LA from plant materials.

LA can be produced from carbohydrate polymers in biomass by acid hydrolysis. Under acidic condition, the carbohydrate polymers are decomposed into a variety of soluble and humiclike products, with LA and formic acid being the final soluble products from hexoses through an intermediate, HMF (*301*). In the process of acid hydrolysis, the reaction temperature and acid concentration play important roles in the final LA yield. Especially, higher LA yield can be obtained at the higher temperature and at the lower acid concentration. It was reported that the LA yield at 200 °C was significantly higher than that at 160 °C with whole kernel grain sorghum as raw materials, and similar results was obtained using corn starches as materials (*302*). At the higher temperature, dilute acid will accelerate the biomass decomposition. Fitzpatrick (*303*) patented a continuous process for producing LA from carbohydrate-containing materials. The process employed a two-stage reaction scheme. The carbohydrate materials were supplied continuously to a first reactor, and hydrolyzed at 210-230 °C and 13-25 s in the presence of 1% to 5% mineral acid. The hydrolysis process produced HMF, which was removed from the first reactor, and supplied to the second reactor. HMF was further hydrolyzed at 195 to 215 °C for 15 to 30 min to produce levulinic acid. The yield was between 60% and 70% of the theoretical yield based on the hexose content of the carbohydrate-containing material (*304*). Ghorpade and Hanna (*286*) described a continuous reactive extrusion technology for levulinic acid production. The slurry of starch mixed with mineral acid was extruded using a twin-screw extruder at 80-150 °C for between 80 and 100 s to produce levulinic acid. Farone and Cuzens (*305*) patented another method the production of levulinic acid and its derivatives. The conversion from biomass to levulinic acid was achieved by hydrolyzing biomass between 40 and 240 °C for 1 to 96 h in the presence of 5 to 90 wt% sulfuric acid. Chang et al. (*306*) reported the effects of temperature, acid concentration, liquid to solid ratio, and reaction time on levulinic acid production from wheat straw using the response surface methodology. Under the optimal conditions of 209.3 °C, 3.5% concentration of acid, 15.6% liquid to solid ratio, and 37.6 min of reaction time, a 19.86% yield of levulinic acid was achieved.

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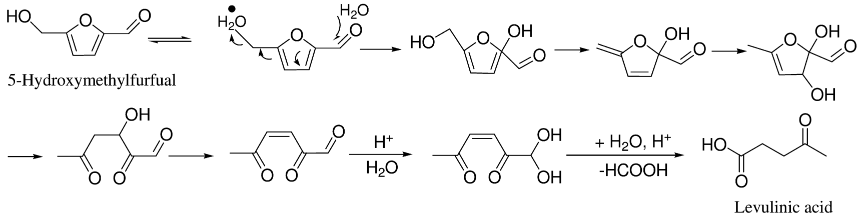
5-Hydroxymethylfurfual
H+
H2O
+ H2O, H+
-HCOOH
Levulinic acid

Figure 7. The reaction mechanism for conversion 5-hydroxymethylfurfural to levulinic acid (291).

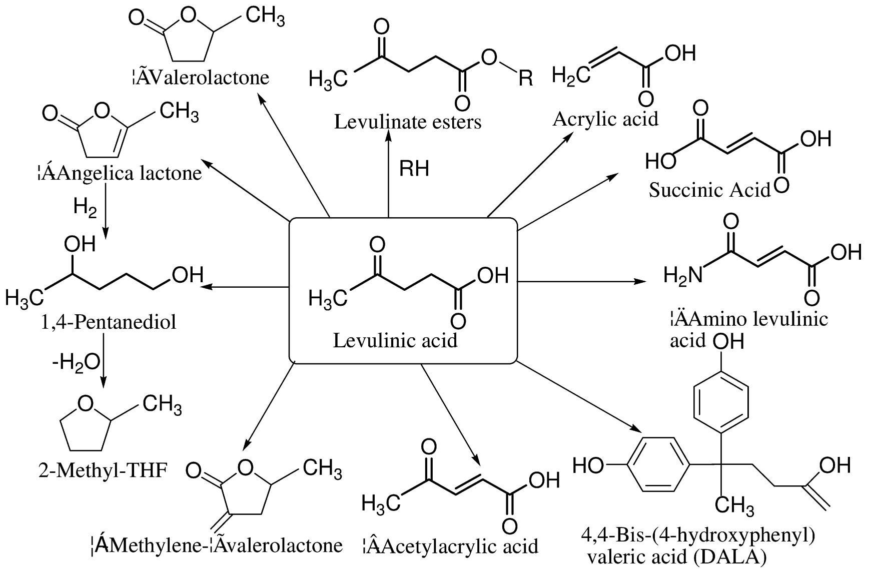
¡ÃValerolactone
Levulinate esters
Acrylic acid
Succinic Acid
¡ÁAngelica lactone
RH
H2
1,4-Pentanediol
-H2O
Levulinic acid
¡ÄAmino levulinic acid
2-Methyl-THF
¡ÁMethylene-¡Ãvalerolactone
¡ÂAcetylacrylic acid
4,4-Bis-(4-hydroxyphenyl) valeric acid (DALA)

Figure 8. Primary derivative from levulinic acid (10, 39).

Levulinic acid contains a ketone carbonyl group (CO) and a carboxylic acid group (COO). These two functional groups make levulinic acid a potentially very versatile building block for the synthesis of various organic (bulk)-chemicals as shown in Figure 8.

One of important reactions involving the carboxylic group of LA is the esterification to produce various esters of LA. Esters of LA are important compounds which are used for flavoring, solvents, and plasticizers. The reaction of LA with primary alcohols, which is a first-order reaction with respect to both reactants, occurs in solutions of LA in respective alcohols even at room temperature (*307*). To obtain high yields of levulinate esters, the reaction is usually carried out in the presence of an acid catalyst, such as sulphuric, polyphosphoric or *p*-toluenesulfonic acid (*308–312*). The use of anion-exchange resin (*313*), 2-halogenopyridinium salts (*314*) and molecular sieves supported by TiO_2/SO_4^{2-} (*315*) can also obtain very good results. Tecaxo and Biofine Inc.

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(*316*) reported that a mixture of 20% ethyl levulinate, 1% of co-additive and 79% diesel can be used in regular diesel engines.

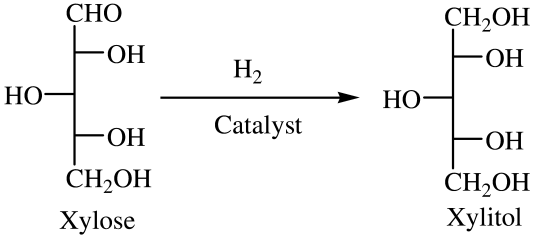
A wide range of interesting LA derivatives is available by nucleophilic additions to the carbonyl group. As an example, in the presence of a metal catalyst and hydrogen gas, LA can react with ammonia or ammonium hydroxide to give 5-methyl-2-pyrrolidone, which is useful intermediate for the pharmaceutical industry (*317*). Diphenolic acid (DPA) is easily prepared from the reaction of LA with two moles of phenol (*318*). DPA can be used for the production of various polymers, lubricants, fire-retardant materials and paints (*319–325*). DPA is of particular interest because it can serve as a replacement for bisphenol A in the production of polycarbonates. The polycarbonates resin market is almost 4 billion lb yr^{-1}, with the product values about \$ 2.40 lb^{-1} (*39*).

The methyl group of LA can be easily halogenated using bromide or chloride to yield organic halides. Therefore, LA undergoes easy chlorination and bromination. For example, δ-aminolevulinic acid (DALA) is prepared by the reaction of 5-bromolevulinic acid with nitrogen-containing nucleophiles, such as sodium azide or potassium phtalimide (*326*, *327*). DALA is a broad spectrum, biodegradable herbicide that shows high activity toward dicotyledonous weeds while showing little activity toward monocotyledonous crops such as corn, wheat, or barley (*282*). In the pharmaceutical industry, DALA has also been used in limited quantities as an active component in photodynamic cancer treatment (*328*). In addition, DALA can be prepared from N-substituted amino acids, and through stepwise buildup of the carbon chain (*329*).

LA can be oxidized to various interesting derivatives. The chemoselectivity is highly depending on the type of oxidant. High-temperature oxidation of LA with oxygen over V_2O_5 gives succinic acid, which is a versatile compound able to undergo a variety of reaction to useful products. LA can also be reduced to 4-hydroxypentanoic acid, which readily dehydrates to γ-valerolactone (GVL). Typical catalysts are platinum oxide, Raney nickel, copper-chromite, rhenium catalysts, rhodium complexes and ruthenium complexes (*330–337*). GVL is known to be useful in industry as a solvent for lacquer, insecticides, adhesives, cutting oils, and brake fluids (*10*). One of the important derivatives of GVL is methyltetrahydrofuran (MTHF), which is a fuel additive with a huge potential market.

Xylitol

Xylitol is currently produced by chemical reduction of xylose derived mainly from wood hydrolysates. The conventional process of xylitol production includes four main steps: acid hydrolysis of plant material, purification of the hydrolysate to either a pure xylose solution or a pure crystalline xylose, hydrogenation of the xylose to xylitol, and crystallization of the xylitol (*338*). The critical step in this process is the purification of the hydrolysate to either a pure xylose solution or a pure crystalline xylose. Ion exchange chromatography is employed to remove salts and charged degradation products, and activated carbon is used to remove color (*339*, *340*). Ion exchange chromatography, however, does not remove or separate the various hemicellulosic sugars. It is a problem because acid hydrolysis releases appreciable amounts of D-galactose, D-mannose and L-arabinose in addition to D-xylose. In order to aviod the high pollution levels and waste-treatment concerns of chemical meothod, the biotechnological process can provide an interesting alternative to the chemical process (*107*).



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Hydrogenation of xylose to xylitol can be facilitated with a multitude of catalysts: several hydrogenation catalysts could in principle be used, such as noble metal (Pt, Pd, Ru) or nickel-based catalysts. The most common catalyst in xylose hydrogenation, promoted Raney nickel, is easily poisoned and deactivated because of the collapse of the pore structure, leaching of the promoter (eg Mo) and accumulation of organic material on the surface. Wisniak et al. (*341*) studied various metallic catalysts in the hydrogenation of glucose, fructose and xylose. They concluded that the activity of the catalytic metals decreases in the order: Ru>Ni>Rh>Pd. The hydrogenation of xylose is economically feasible at elevated temperatures and pressures: a high enough temperature (80-130 °C) is required to get a sufficient reaction rate but too high a temperature leads to formation of undesired by-product. Since the solubility of hydrogen in the aqueous phase is rather low, an elevated pressure is a necessity (generally 40 bar or higher). Hydrogenation of xylose on nickel is selective, provided that the reaction temperature is sufficiently low (less than 130 °C) and the pH is maintained at neutral, or moderately acidic. The by-products of the hydrogenation are D-xylulose, D-arabinitol, furfural and D-xylonic acid. Formation of by-products, such as D-xylulose and furfural, is favoured by shortage of hydrogen on the catalyst surface (*342*).

There are several compounds derived from xylitol, such as xylaric acid, lactic acid, glycerol, ethylene glycol, propylene glycol, and mixture of hydroxyl furans (Figure 9). Xylaric acid can be obtained by the selective oxidation of xylitol. The oxidation chemistry has been demonstrated for the oxidation of sorbitol to glucaric acid but with low efficiency. The conversion of xylitol to glycols, namely propylene glycol and ethylene glycol is based on hydrogenolysis. It has been demonstrated that the reasonable yields (80%) of ethylene glycol and propylene glycol can be achieved from xylitol (*39*). C5 sugars such as xylose and arabinose have the potential to be an outstanding building block for commodity chemicals.

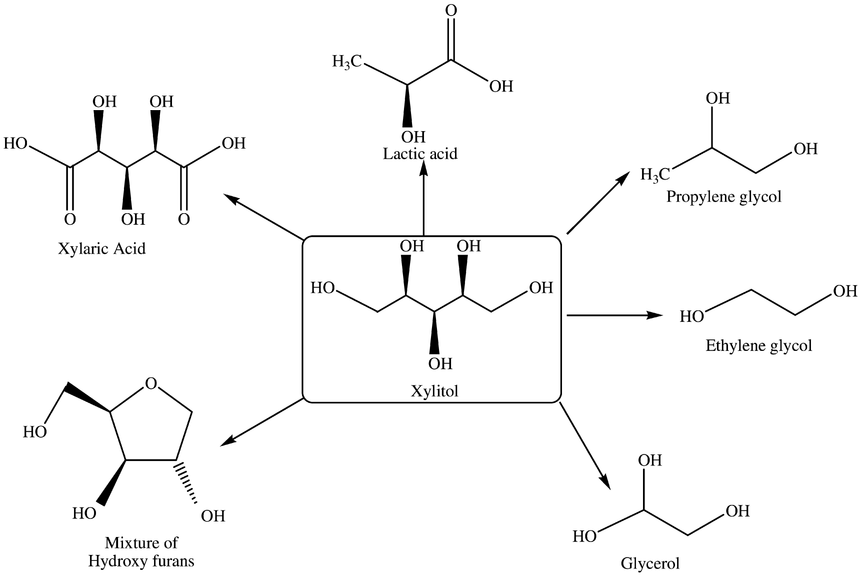

Figure 9. Primary derivative from levulinic acid (39).

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References

1. Bender, M. *Resour. Conserv. Recy.* **2000**, *30*, 49–58.
2. Demirbas, M. F. *Energy Sources, Part A* **2006**, *28*, 1181–1188.
3. Naik, S. N.; Goud, V. V.; Rout, P. K.; Dalai, A. K. *Renewable Sustainable Energy Rev.* **2010**, *14*, 578–597.
4. Cherubini, F. *Energy Convers. Manage.* **2010**, *51*, 1412–1421.
5. Koutinas, A. A.; Wang, R. H.; Webb, C. *Biofuels, Bioprod. Bioref.* **2007**, *1*, 24–38.
6. Eggersdorfer, M.; Meijer, J.; Eckes, P. *FEMS Microbiol. Rev.* **1992**, *103*, 355–364.
7. van Haveren, J.; Wageningen, B. V.; Scott, E. L.; Sanders, J. *Biofuels, Bioprod. Bioref.* **2008**, *2*, 41–57.
8. Okkerse, C.; van Bekkum, H. *Green Chem.* **1999**, *1*, 107–114.
9. Kamm, B.; Gruber P. R.; Kamm, M. *Biorefineries-Industrial Process and Products, Status Quo and Future Directions*; Wiley-VCH: Verlag & Co. KGaA, 2005; Vol. 1, pp 5−18.
10. Corma, A.; Iborra, S.; Velty, A. *Chem. Rev.* **2007**, *107*, 2411–2250.

Downloaded by DUKE UNIV on June 17, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch009

11. Fang, J. M.; Sun, R. C.; Tomkinson, J.; Fowler, O. *Carbohydr. Polym.* **2000**, *41*, 379–387.
12. Alén, R. In *Forest Products Chemistry*; Stenius, P., Ed.; Fapet Oy: Helsinki, 2000; pp 12−57.
13. Pereira, H., Graça, J., Rodrigues, J. C. In *Wood Quality and Its Biological Basis*; Barnett, J. R., Jeronimidis, G., Eds.; Blackwell Publishing: Oxford, 2003; pp 53−86.
14. Shibuya, N.; Iwasaki, T. *Phytochemistry* **1985**, *24*, 285–289.
15. Brillouet, J. M.; Joseleau, J. P.; Utille, J. P.; Lelievre, D. *J. Agric. Food Chem.* **1982**, *30*, 488–495.
16. Wende, G.; Fry, S. C. *Phytochemistry* **1997**, *44*, 1011–1018.
17. Timell, T. E. *Adv. Carbohydr. Chem.* **1964**, *19*, 247–302.
18. Bacon, J. S. D.; Gordon, A. H.; Morries, E. J. *Biochem. J.* **1975**, *149*, 485–487.
19. de Vries, R. P.; Visser, J. *Microbiol. Mol. Biol. Rev.* **2001**, *65*, 497–522.
20. Scheller, H. V.; Ulvskov, P. *Annu. Rev. Plant Biol.* **2010**, *61*, 263–289.
21. Maruyama, K.; Goto, C.; Numata, M.; Suzuki, T.; Nakagawa, Y.; Hoshino, T.; Uchiyama, T. *Phytochemistry* **1996**, *41*, 1309–1314.
22. Sims, I. M.; Munro, S. L. A.; Currie, G.; Craik, D.; Bacic, A. *Carbohydr. Res.* **1996**, *293*, 147–172.
23. Stone, B. A.; Clarke, A. E. *Chemistry and Biology of (1→3)-β-Glucans*; La Trobe University Press: Bundoora, 1992; pp 1−116.
24. Kondo, T.; Hiroi, T.; Mizuno, K.; Kato, T. *Can. J. Plant Sci.* **1990**, *70*, 193–201.
25. Eriksson, Ö.; Lindgren, B. *Svensk Papperstidn* **1977**, *80*, 59–63.
26. Freudenberg, K. *Science* **1965**, *148*, 595–600.
27. Lam, T. B.; Liyama, K.; Stone, B. A. *Phytochemistry* **1992**, *31*, 2655–2658.
28. Sun, R. C.; Tomkinson, J. *Eur. Polym. J.* **2003**, *39*, 751–759.
29. Sun, R. C.; Sun, X. F. *Polym. Degrad. Stabil.* **2002**, *78*, 295–303.
30. Ford, C. W. *Carbohydr. Res.* **1989**, *190*, 137–144.
31. Taylor, J. G.; Haigler, C. H. *Acta Bot. Neerl.* **1993**, *42*, 153–163.
32. Bao, W.; O′malley, D. M.; Sederoff, R. R. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 6604.
33. Gregory, A. C. E.; O′Connell, A. P.; Bolwell, G. P. *Biotechnol. Genet. Eng. Rev.* **1998**, *15*, 439–455.
34. Stacey, N. J.; Roberts, K.; Carpita, N. C.; Wells, B.; McCann, M. C. *Plant J.* **1995**, *8*, 891–906.
35. Sjöström. E. In *Wood Chemistry, Fundamental and Applications*; Academic Press: New York, 1981.
36. Girio, F. M.; Fonseca, C.; Carvalheiro, L. C.; Duarte, S.; Marques, R. B. *Bioresour. Technol.* **2010**, *101*, 4775–4800.
37. Wright, L.; Boundy, B.; Perlack, B.; Davis, S.; Saulsbury. B. *Biomass Energy Data Book*; U S Department of Energy (DOE): Oak Ridge, TN, 2006.
38. Carvalheiro, F.; Duarte, L. C.; Girio, F. M. *J. Sci. Ind. Res.* **2008**, *67*, 849–864.
39. US Department of Energy, Office of Scientific and Technical Information, No. DOE/GO-102004-1992. http:// www.nrel.gov/docs/fy04osti/35523.pdf.

Downloaded by DUKE UNIV on June 17, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch009

40. Bozell, J. J.; Petersen, G. *Green Chem.* **2010**, *12*, 539–554.
41. Lichtenthaler, F. W.; Peters, S. *C. R. Chim.* **2004**, *7*, 65–90.
42. Lichtenthaler, F. W. *Carbohydr. Res.* **1998**, *313*, 69–89.
43. Lichtenthaler, F. W. *Acc. Chem. Res.* **2002**, *35*, 728–737.
44. Chandel, A. K.; Singh, O. V.; Rao L. V. In *Sustainable Biotechnology*; Singh, O. V., Harvey, S. P., Eds.; Springer Dordrechet: Heidelberg, London, New York, 2010; pp 63−81.
45. Lynd, L. R.; Laser, M. S.; Bransby, D.; Dale, B. E.; Davison, B.; Hamilton, R.; Himmel, M.; Keller, M.; McMillan, J. D.; Sheehan, J.; Wyman, C. E. *Nat. Biotechnol.* **2008**, *26*, 169–172.
46. Saha, B. C. *J. Ind. Microbiol. Biotechnol.* **2003**, *30*, 279–291.
47. Gray, K. A.; Zhao, L.; Emptage, M. *Curr. Opin. Chem. Biol.* **2006**, *10*, 1–6.
48. Viikari, L.; Tenkanen, M.; Buchert, J.; Ratto, M.; Bailey, M.; Siikaaho-Aho, M. In *Biotechnology in Agriculture*; Saddler, J. N., Ed.; CAB International: 1993; pp 131−182
49. Wong, K. K. Y.; Tan, L. U. L.; Saddler, J. N. *Microbiol. Rev.* **1988**, *52*, 305–317.
50. Zeikus, J. G.; Lee, C.; Lee, Y. E.; Saha, B. C. In *Enzymes in Biomass Conversion*; Leatham G. F., Himmel, M. E., Eds.; ACS Symposium Series 460; American Chemical Society: Washington, D C, 1991; pp 36−51.
51. Beg, Q. K.; Kapoor, M.; Mahajan, L.; Hoondal, G. S. *Appl. Microbiol. Biotechnol.* **2001**, *56*, 326–338.
52. Biely, P. *Trends Biotechnol.* **1985**, *3*, 286–290.
53. Sun, R. C.; Sun, X. F.; Tomkinson, J. In *Hemicelluloses: Science and Technolgy*; Gatenholm, P., Tenkanen, M., Eds.; ACS Syposium Sereis 864; American Chemical Society: Washington, DC, 2003; pp 2−22.
54. Puls, J.; Schmidt, O.; Granzow, C. *Enzyme Microb. Technol.* **1987**, *9*, 83–88.
55. Subramaniyan, S.; Prema, P. *Crit. Rev. Biotechnol.* **2002**, *22*, 33–64.
56. Knob, A.; Terrasan, C. R. F.; Carmona, E. C. *World J. Microbiol. Biotechnol.* **2010**, *26*, 389–407.
57. Collins, T.; Gerday, C.; Feller, G. *FEMS Microbiol. Rev.* **2005**, *29*, 3–23.
58. Polizeli, M. L. T. M.; Rizzatti, A. C. S.; Monti, R.; Terenzi, H. F.; Jorge, J. A.; Amorim, D. S. *Appl. Microbiol. Biotechnol.* **2005**, *67*, 577–591.
59. Sunna, A.; Antranikian, G. *Crit. Rev. Biotechnol.* **1997**, *17*, 39–67.
60. Saha, B. C. *Biotechnol. Adv.* **2000**, *18*, 403–423.
61. Saha, B. C.; Bothast, R. *J. Appl. Environ. Microbiol.* **1998**, *64*, 216–220.
62. Sozzi, G. O.; Greve, L. C.; Prody, G. A.; Labavitch, J. M. *Plant Physiol.* **2002**, *129*, 1330–1340.
63. Siika-aho, M.; Tenkanen, M.; Buchert, J.; Puls, J.; Viikari, L. *Enzyme Microb. Technol.* **1994**, *16*, 813–819.
64. Shallom, D.; Shoham, Y. *Curr. Opin. Microbiol.* **2003**, *6*, 219–228.
65. Biely, P.; Vrsanska, M.; Tenkanen, M.; Kluepfel, D. *J. Biotechnol.* **1997**, *57*, 151–166.
66. Grohmann, K.; Mitchell, D. J.; Himmel, M. E.; Dale, B. E.; Schroeder, H. A. *Appl. Biochem. Biotech.* **1989**, *20/21*, 45–61.
67. Puls, J.; Schuseil, J. In *Hemicelluloses and Hemicellulases*; Coughlan, M. P., Hazlewood, G. P., Eds.; Portland Press: London, 1993; pp 1–27.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch009

68. Lee, S. F.; Forsberg, C. W. *Can. J. Microbiol.* **1987**, *33*, 1011–1016.
69. Belancic, A.; Scarpa, J.; Peirano, A.; Diaz, R.; Steiner, J.; Eyzaguirre, J. *J. Biotechnol.* **1995**, *41*, 71–79.
70. Elegir, G.; Szakacs, M.; Jeffries, T. W. *Appl. Environ. Microbiol.* **1994**, *60*, 2609–2615.
71. Wubah, D. A.; Akin, D. E.; Borneman, W. S. *Crit. Rev. Microbiol.* **1993**, *19*, 99–115.
72. Matte, A.; Forsberg, C. W. *Appl. Environ. Microbiol.* **1992**, *58*, 157–168.
73. Talebnia, F.; Karakashev, D.; Angelidaki, I. *Bioresour. Technol.* **2010**, *101*, 4744–4753.
74. Wiegel, J.; Ljungdahl, L. G. *Crit. Rev. Biotechnol.* **1986**, *3*, 39–108.
75. Desai, S. G.; Guerinot, M. L.; Lynd, L. R. *Appl. Microbiol. Biotechnol.* **2004**, *65*, 600–605.
76. Hahn-Hägerdal, B.; Galbe, M.; Gorwa-Grauslund, M. F.; Liden, G.; Zacchi, G. *Trends Biotechnol.* **2006**, *24*, 549.
77. Gamage, J.; Lam, H.; Zhang, Z. S. *J. Biobased Mater. Bioenergy* **2010**, *4*, 3–11.
78. Dien, B. S.; Cotta, M. A.; Jeffries, T. W. *Appl. Microbiol. Biotechnol.* **2003**, *63*, 258–266.
79. Swings, J.; DeLey, J. *Bacteriol. Rev.* **1977**, *41*, 1–46.
80. Lee, K. J.; Tribe, D. E.; Rogers, P. L. *Biotechnol. Lett.* **1979**, *1*, 421–426.
81. Rogers, P. L.; Lee, K. J.; Tribe, D. E. *Biotechnol. Lett.* **1979**, *1*, 165–170.
82. Mohaghgheghi, A.; Evans, K.; Chou, Y. C.; Zhang, M. *Appl. Biochem. Biotechnol.* **2002**, *98*, 885–898.
83. Balat, M.; Balat, H.; Oz, C. *Prog. Energy Combust. Sci.* **2008**, *34*, 551–573.
84. Claassen, P. A. M.; Lopez Contreras, A. M.; Sijtsma, L.; Weusthuis, R. A.; Van Lier, J. B.; Van Niel, E. W. J.; Stams, A. J. M.; De Vries, S. S. *Appl. Microbiol. Biotechnol.* **1999**, *52*, 741–755.
85. Jorgensen, H. *Appl. Biochem. Biotechnol.* **2009**, *153*, 44–57.
86. Hahn-Hägerdal, B.; Karhumaa, K.; Fonseca, C.; Spencer-Martins, I.; Marie, F. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 937–953.
87. Olsson, L.; Linden, T.; Hahn-Hägerdal, B. *Appl. Biochem. Biotechnol.* **1992**, *34–35*, 359–368.
88. Hahn-Hägerdal, B.; Jeppsson, H.; Olsson, L.; Mohagheghi, A. *Appl. Microbiol. Biotechnol.* **1994**, *41*, 62–72.
89. Chandel, A. K.; Chan, E. S.; Rudravaram, R.; Narasu, M. L.; Rao, L. V.; Pogaku, R. *Biotechnol. Mol. Biol. Rev.* **2007**, *2*, 14–32.
90. Lin, Y.; Tanaka, S. *Appl. Microbiol. Biotechnol.* **2006**, *69*, 627–642.
91. Karhumaa, K.; Sanchez, R. G.; Hahn-Hägerdal, B.; Gorwa-Grauslund, M. F. *Microb. Cell Fact.* **2007**, *6*, 5.
92. Jeffries, T. W.; Van Vleet, J. R. *FEMS Yeast Res.* **2009**, *9*, 793–807.
93. Ozcan, S.; Kotter, P.; Ciriacy, M. *Appl. Microbiol. Biotechnol.* **1991**, *36*, 190–195.
94. Wu, J. F.; Lastick, S. M.; Updegraff, D. M. *Nature* **1986**, *321*, 887–888.
95. Panagiotou, G.; Christakopoulos, P.; Grotkjaer, T.; Olsson, L. *Metab. Eng.* **2006**, *8*, 474–482.

Downloaded by DUKE UNIV on June 17, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch009

96. Boxma, B.; Voncken, F.; Jannink, S.; van Alen, T.; Akhmanova, A.; van Weelden, S. W.; van Hellemond, J. J.; Ricard, G.; Huynen, M.; Tielens, A. G.; Hackstein, J. H. E. *Mol. Microbiol.* **2004**, *51*, 1389–1399.
97. Singh, A.; Kumar, P. K. R.; Schügerl, K. *Adv. Biochem. Eng./Biotechnol.* **1992**, *45*, 29–55.
98. Hahn-Hägerdal, B.; Wahlborm, C. F.; Gardonyi, M.; van Zyl, W. H.; Cordero Otero, R. R.; Jonsson, L. J. *Adv. Biochem. Eng. Biotechnol.* **2001**, *73*, 53–84.
99. Ho, N. W. Y.; Chen, Z.; Brainard, A. P. *Appl. Environ. Microbiol.* **1998**, *64*, 1852–1856.
100. Ingram, L. O.; Alterhum, F.; Ohta, K.; Beall, D. S. In *Developments in Industrial Microbiology*; Pierce, G. E., Ed.; Elsevier Science: New York, 1990; Vol. 31, pp 21−30.
101. Zhang, M.; Eddy, C.; Deanda, K.; Finkelstein, M.; Picataggio, M. *Science* **1995**, *267*, 240–243.
102. Ingram, L. O.; Conway, T.; Clark, D. P.; Sewell, G. W.; Preston, J. F. *Appl. Environ. Microbiol.* **1987**, *53*, 2420–2425.
103. Burchhardt, G.; Ingram, L. O. *Appl. Environ. Microbiol.* **1992**, *58*, 1128–1133.
104. Kotter, P.; Ciriacy, M. *Appl. Microbiol. Biotechnol.* **1993**, *38*, 776–783.
105. Ylikahri, R. *Adv. Food Res.* **1979**, *25*, 159–180.
106. Parajó, J. C.; Dominguez, H.; Dominguez, J. M. *Bioresour. Technol.* **1998**, *65*, 191–201.
107. Winkelhausen, E.; Kuzmanova, S. *J. Ferment. Bioeng.* **1998**, *86*, 1–14.
108. Lima, L. H. A.; Felipe, M. D. D.; Vitolo, M.; Torres, F. A. G. *Appl. Microbiol. Biotechnol.* **2004**, *65*, 734–738.
109. Parajo, J. C.; Dominguez, H.; Dominguez, J. M. *Bioresour. Technol.* **1998**, *65*, 203–212.
110. Yoshitake, J.; Obiwa, H.; Shimamura, M. *Agric. Biol. Chem.* **1971**, *35*, 905–911.
111. Yoshitake, J.; Ishizaki, H.; Shimamura, M.; Imai, T. *Agric. Biol. Chem.* **1973**, *37*, 2261–2267.
112. Yoshitake, J.; Shimamura, M.; Ishizaki, H.; Irie, Y. *Agric. Biol. Chem.* **1976**, *40*, 1493–1503.
113. Izumori, K.; Tuzaki, K. *J. Ferment. Technol.* **1988**, *66*, 33–36.
114. Rangaswamy, S.; Agblevor, F. A. *Appl. Microbiol. Biotechnol.* **2002**, *60*, 88–93.
115. Chiang, C.; Knight, S. G. *Nature* **1960**, *188*, 79–81.
116. Dahija, J. S. *Can. J. Microbiol.* **1991**, *37*, 14–18.
117. Ueng, P. P.; Gong, C. S. *Enzyme Microb. Technol.* **1982**, *4*, 169–171.
118. Zhao, X.; Gao, P.; Wang, Z. *Appl. Biochem. Biotechnol.* **1998**, *70–72*, 405–414.
119. Sampaio, F. C.; da Silveira, W. B.; Chaves-Alves, V. M.; Passos, F. M. L.; Coelho, J. L. C. *Braz. J. Microbiol.* **2003**, *34*, 325–328.
120. Rao, R. S.; Jyothi, C. P.; Prakasham, R. S.; Sarma, P. N.; Rao, L. V. *Bioresour. Technol.* **2006**, *97*, 1974–1978.
121. Barbosa, M. F. S.; Medeiros, M. B.; Mancilha, I. M.; Schneider, H.; Lee, H. *J. Ind. Microbiol.* **1988**, *3*, 241–251.

Downloaded by DUKE UNIV on June 17, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch009

Downloaded by DUKE UNIV on June 17, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch009

122. Oh, D. K.; Kim, S. Y.; Kim, J. H. *Biotechnol. Bioeng.* **1998**, *58*, 440–444.
123. Converti, A.; Perego, P.; Sordi, A,; Torre, P. *Appl. Biochem. Biotechnol.* **2002**, *101*, 15–29.
124. Girio, F. M.; Roseiro, J. C.; Sa-Machado, P.; Duarte-Reis, A. R.; Amaral-Collaco, M. T. *Enzyme Microb. Technol.* **1994**, *16*, 1074–1078.
125. Rivas, B.; Paolo, T.; Dominguez, J. M.; Converti, A. *Biotechnol. Bioeng.* **2009**, *102*, 1062–1073.
126. Saha, B. C., Bothast, R. J. In *Fuels and Chemicals from Biomass*; Saha, B. C., Woodward, J., Eds.; ACS Symposium Series 666; American Chemical Society: Washington, DC, 1997; pp 307−309.
127. Taylor, K. B.; Beck, M. J.; Huang, D. H.; Sakai, T. T. *J. Ind. Microbiol.* **1990**, *6*, 29–41.
128. Smiley, K. L.; Bolen, P. L. *Biotechnol. Lett.* **1982**, *4*, 607–610.
129. Skoog, K.; Hahn-Hägerdal, B. *Enzyme Microbiol. Technol.* **1988**, *10*, 66–79.
130. Girio, F. M.; Peito, M. A.; Amaral-Collaqo, M. T. In *Biomass for Energy and Industry*; Grassi, G., Gosse, G., dos Santos, G., Eds.; Elsevier Applied Science: London, 1990; Vol. 2.
131. Prior, B. A.; Kilian, S. G.; Du Preez, J. C. *Process Biochem.* **1989**, *24*, 21–32.
132. Girio, F. M.; Amaro, C.; Azinheira, H.; Pelica, F.; Amaral-Collaco, M. T. *Bioresour. Technol.* **2000**, *71*, 245–251.
133. Parajo, J. C.; Dominguez, H.; Dominguez, J. M. *Enzyme Microb. Technol.* **1997**, *21*, 18–24.
134. Granstrom, T. B.; Aristidou, A. A.; Jokela, J.; Leisola, M. *Biotechnol. Bioeng.* **2000**, *70*, 197–207.
135. Nobre, A.; Lucas, T.; Leao, C. *Appl. Environ. Microbiol.* **1999**, *65*, 3594–3598.
136. Sampaio, F. C.; Torre, P.; Passos, F. M. L.; Perego, P.; Passos, F. J. V.; Converti, A. *Biotechnol. Prog.* **2004**, *20*, 1641–1650.
137. Felipe, M. G. A. In *Lignocellulose Biodegradation*; Saha, B. C., Hayashi, K., Eds; ACS Symposium Series 889; American Chemical Society: Washington, DC, 2004; pp 300–315.
138. Kumar, P. K. R.; Singh, A.; Schiigerl, K. *Proc. Biochem.* **1991**, *26*, 209–216.
139. Schiigerl, K. *Bioreaction Engineering*; John Wiley & Sons: New York, 1987; Vol. 1.
140. Vandeska, E.; Amartey, S.; Kuzmanovat, S.; Jeffries, T. W. *Process Biochem.* **1996**, *3*, 265–270.
141. Garg, S. K.; Jain, A. *Bioresour. Technol.* **1995**, *51*, 103–109.
142. Flickinger, M. C. *Biotechnol. Bioeng.* **1980**, *22*, 27–48.
143. Yu, E. K.; Saddler, J. N. *Biotechnol. Lett.* **1982**, *4*, 121–126.
144. Villet, R. Biotechnology for producing chemicals from biomass; Solar Energy Research Institute: Golden, Colorado, SERI/TR-621-754, 1981; Vol. 2.
145. Soltys, K. A.; Batta, A. K.; Koneru, B. *J. Surg. Res.* **2001**, *96*, 30–34.
146. Bartowsky, E. J.; Henschke, P. A. *Int. J. Food Microbiol.* **2004**, *96*, 235–252.
147. Kluyver, A. J.; Donker, H. J. L.; Hooft, F. V. *Biochem. Z.* **1925**, *161*, 361–378.

148. Fulmer, E. I.; Christensen, L. M.; Kendall, A. R. *Ind. Eng. Chem.* **1933**, *25*, 798–800.
149. Kosaric, N.; Velikonja, J. *FEMS Microbiol. Rev.* **1995**, *16*, 111–142.
150. Yu, E. K. C.; Saddler, J. N. *Appl. Environ. Microbiol.* **1982**, *44*, 777–784.
151. Saddler, J. N.; Yu, E. C. K.; Mes-Hartree, M.; Levitin, N.; Brownell, H. H. *Appl. Environ. Microbiol.* **1983**, *45*, 153–160.
152. Celińska, E.; Grajek, W. *Biotechnol. Adv.* **2009**, *27*, 715–725.
153. Hespell, R. B. *Curr. Microbiol.* **1996**, *32*, 291.
154. Nakashimada, Y.; Kanai, K.; Nishio, N. *Biotechnol. Lett.* **1998**, *20*, 1133–1138.
155. Beronio, P. B.; Tsao, G. T. *Biotechnol. Bioeng.* **1993**, *42*, 1263.
156. Ji, X. J.; Huang, H.; Du, J.; Zhu, J. G.; Ren, L. J.; Hu, N.; Li, S. *Bioresour. Technol.* **2009**, *100*, 3410–3414.
157. Ji, X. J.; Huang, H.; Du, J.; Zhu, J. G.; Ren, L. J.; Li, S.; Nie, Z. K. *Bioresour. Technol.* **2009**, *100*, 5214–5218.
158. Ma, C. Q.; Wang, A. L.; Qin, J. Y.; Li, L. X.; Ai, X. L.; Jiang, T. Y.; Tang, H. Z.; Xu, P. *Appl. Microbiol. Biotechnol.* **2009**, *82*, 49–57.
159. Converti, A.; Perego, P.; Del Borghi, M. *Biotechnol. Bioeng.* **2003**, *82*, 370–377.
160. Zeng, A. P.; Biebl, H.; Deckwer, W. D. *Appl. Microbiol. Biotechnol.* **1990**, *33*, 264–268.
161. Zheng, J. L.; Jia, J.; Wei, X. X.; Shen, X. W.; Chen, G. Q. *Appl. Microbiol. Biotechnol.* **2010**, *87*, 2001–2009.
162. Moes, J.; Griot, M.; Keller, J.; Heinzle, E.; Dunn, I. J.; Bourne, J. R. *Biotechnol. Bioeng.* **1985**, *27*, 482–489.
163. Grover, B. S.; Garg, S. K.; Verma, J. *World J. Microbiol. Biotechnol.* **1990**, *6*, 328–332.
164. Voloch, M.; Jansen, N. B.; Ladish, M. R.; Tsao, G. T.; Narayan, R.; Rodwell, V. W. In *Comprehensive Biotechnology: The Principles, Applications and Regulations of Biotechnology in Industry, Agriculture and Medicine*; Blanch, H. W., Drew, S., Wang, D. I. C., Eds.; Pergamon/Elsevier: Oxford, 1985; pp 933−944.
165. Perego, P.; Converti, A.; Del Borghi, A.; Canepa, P. *Bioprocess Eng.* **2000**, *23*, 613–620.
166. Perlman, D. *Ind. Eng. Chem.* **1944**, *36*, 803–804.
167. Syu, M. J. *Appl. Microbiol. Biotechnol.* **2001**, *55*, 10–18.
168. Olson, B. H.; Johnson, M. J. *J. Bacteriol.* **1948**, *55*, 209–222.
169. Laube, V. M.; Groleau, D.; Martin, S. M. *Biotechnol. Lett.* **1984**, *6*, 535–540.
170. Qin, J. Y.; Xiao, Z. J.; Ma, C. Q.; Xie, N. Z.; Liu, P. H.; Xu, P. *Chinese J. Chem. Eng.* **2006**, *14*, 132–136.
171. de Mattos, M. J. T.; Neijssel, O. M. *J. Biotechnol.* **1997**, *59*, 117–126.
172. Toivari, M. H.; Maaheimo, H.; Penttila, M.; Ruohonen, L. *Appl. Microbiol. Biotechnol.* **2010**, *3*, 731–739.
173. Drumright, R. E.; Gruber, P. R.; Henton, D. E. *Adv. Mater.* **2000**, *12*, 1841–1846.
174. Åkerberg, C.; Zacchi, G. *Bioresour. Technol.* **2000**, *75*, 119–126.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch009

175. Maas, R. H. W.; Bakker, R. R.; Eggink, G.; Weusthuis, R. A. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 861–868.
176. Maas, R. H. W.; Springer, J.; Eggink, G.; Ruud, A. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 569–578.
177. Yoshida, S.; Okano, K.; Tanaka, T.; Ogino, C.; Kondo, A. *J. Biosci. Bioeng.* **2009**, *108*, S41–S56.
178. Anupama, P.; Ravindra, L. *Biotechnol. Adv.* **2000**, *18*, 459–479.
179. Voltolina, D.; Gomez-Villa, H.; Correa, G. *Bioresour. Technol.* **2005**, *96*, 359–362.
180. Kuhad, R. C.; Singh, A.; Tripathi, K. K.; Saxena, R. K.; Eriksson, K. L. *Nutr. Rev.* **1997**, *55*, 65–75.
181. Meyer, P. S.; Du Preez, J. C.; Kilian, S. G. *Biotechnol. Bioeng.* **1992**, *40*, 353–358.
182. Brady, J. E.; Russell, J. W.; Holum, J. R. *Chemistry: Matter and Its Changes*; John Wiley: New York, 2000; pp 1055−1057.
183. Win, D. T. *Aust. J. Technol.* **2005**, *8*, 185–190.
184. Montane, D.; Salvado, J.; Torras, C.; Farriol, X. *Biomass Bioenergy* **2002**, *22*, 295–304.
185. Demirbas, A. *Energy Sources, Part A* **2006**, *28*, 157–165.
186. Mamman, A. S.; Lee, J. M.; Kim, Y. C.; Hwang, I. T.; Park, N. J.; Hwang, Y. K.; Chang, J. S.; Hwang, J. S. *Biofuels, Bioprod. Bioref.* **2008**, *2*, 438–454.
187. Wyman, C. E. *Bioresour. Technol.* **1994**, *50*, 3–16.
188. Vazquez, M.; Martha, O.; Tellez-Luis, S. J.; Ramırez, J. A. *Bioresour. Technol.* **2007**, *98*, 3053–3060.
189. Nguyen, Q. A.; Tucker, M. P.; Keller, F. A.; Eddy, F. P. *Appl. Biochem. Biotechnol.* **2000**, *84–86*, 561–576.
190. Herrera, A.; Tellez-Luis, S. J.; Ramırez, J. A.; Vazquez, M. *J. Cereal Sci.* **2003**, *37*, 267–274.
191. Herrera, A.; Tellez-Luis, S. J.; Gonzalez-Cabriales, J. J.; Ramirez, J. A.; Vazquez, M. *J. Food Eng.* **2004**, *63*, 103–109.
192. Franz, R.; Erckel, R.; Riehm, T.; Woernle, R.; Deger, H. M. *Energy from Biomass*; Applied Science Publishers: London, 1982; pp 873–878.
193. Conner, A. H. *Wood Fiber Sci.* **1984**, *16*, 268–277.
194. Gamez, S.; Ramırez, J. A.; Garrote, G.; Vazquez, M. *J. Agric. Food Chem.* **2004**, *52*, 4172–4177.
195. Carrasco, F.; Roy, C. *Wood Sci. Technol.* **1992**, *26*, 189–208.
196. Oshima, M. *Wood Chemistry Process Engineering Aspects*; Noye Development Corp: New York, 1965.
197. Zeittsch, K. J. *The Chemistry and Technology of Furfural and Its Many Byproducts*; Elsevier Publications: Netherlands, 2000; Vol. 13.
198. Kottke, R. H. In *Kirk-Othmer Encyclopedia of Chemical Technology*; Kroschwitz, J., Howe-Grant, M., Eds.; John Wiley and Sons: New York, 1998; Volume supplement.
199. Antal, M. J.; Leesoboom, T.; Mok, W. S.; Richards, G. N. *Carbohydr. Res.* **1991**, *217*, 71–85.
200. Sangarunlert, W.; Piumsomboon, P.; Ngamprasertsith, S. *Korean J. Chem. Eng.* **2007**, *24*, 936–941.

Downloaded by DUKE UNIV on June 17, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch009

201. Mansilla, H. D.; Baeza, J.; Urzua, S.; Maturana, G.; Villasenor, J.; Duran, N. *Bioresour. Technol.* **1998**, *66*, 189–193.
202. Lavarack, B. P.; Griffin, G. J.; Rodman, D. *Biomass Bioenergy* **2002**, *23*, 367–380.
203. Demirbas, A. *Energy Convers. Manage.* **2001**, *42*, 279–294.
204. Sako, T.; Sugeta, T.; Nakazawa, N.; Otake, K.; Sato, M.; Ishihara, K.; Kato, M. *Fluid Phase Equilib.* **1995**, *108*, 293–303.
205. Gamse, T.; Marr, R. *Sep. Sci. Technol.* **1997**, *32*, 355–371.
206. Sihvonen, M.; Jarvenpaa, E.; Hietaniemi, V.; Huopalahti, R. *Trends Food Sci. Technol.* **1999**, *10*, 217–222.
207. Sako, T.; Sugeta, T.; Nakazawa, N.; Okubo, T.; Sato, M.; Taguchi, T.; Hiaki, T. *J. Chem. Eng. Jpn.* **1992**, *25*, 372–377.
208. Sangarunlert, W.; Piumsomboon, P.; Ngamprasertsith, S. *Korean J. Chem. Eng.* **2007**, *24*, 936–941.
209. Moreau, C.; Belgacem, M. N.; Gandini, A. *Top. Catal.* **2004**, *27*, 11–30.
210. Moreau, C.; Durand, R.; Peyron, D.; Duhamet, J.; Rivalier, P. *Ind. Crops Prod.* **1998**, *7*, 95–99.
211. Moreau, C. *Agro Food Ind. Hi-Tech* **2002**, *1*, 17–26.
212. Dias, A. S.; Pillinger, M.; Valente, A. A. *J. Catal.* **2005**, *229*, 414–423.
213. Lansalot-Matras, C.; Moreau, C. *Catal. Commun.* **2003**, *4*, 517–520.
214. Mickillip, W. J. In *Ullmann's Encyclopedia of Industrial Chemistry*; Elvers, B., Hawkins, S., Ravenscroft, M., Rounsaville, J. F., Schulz G., Eds.; Verlagasgesellschaft mbH: Federal Republic of Germany, 1989; Vol. A12, pp 125.
215. Othmer, K. In *Encyclopedia of Chemical Technology*; Bushey, G. J., Eastman, C. I., Klingsberg, A., Spiro L., Eds.; John Wiley & Sons, Inc., New York, 1980; pp 510.
216. Bauer, K.; Garbe, D. *Common Fragrance and Flavor Materials*; VCH Publishers: Kyoto, Japan, 1985.
217. Li, M. S.; Ma, W. Z. *Hebai Ind.* **1999**, *6*, 3.
218. Cottier, L.; Descotes, G. *Trends Heterocycl. Chem.* **1991**, *2*, 233–248.
219. Lewkowski, J. *Arkivoc* **2001**, *i*, 17–54.
220. Ulbricht, R. J.; Northup, S. J.; Thomas, J. A. *Fundam. Appl. Toxicol.* **1984**, *4*, 843–853.
221. Dull, G. *Chem.-Ztg.* **1895**, *19*, 216.
222. Kiermayer, J. *Chem.-Ztg.* **1895**, *19*, 1003.
223. Shaw, P. E.; Tatum, J. H.; Berry, R. E. *Carbohydr. Res.* **1967**, *5*, 266–273.
224. Van Dam, H. E.; Kieboom, A. P. G.; Van Bekkum, H. *Starch/Stärke* **1986**, *38*, 95–101.
225. Cottier, L.; Descotes, G.; Neyret, C.; Nigay, H. *Ind. Aliment. Agric.* **1989**, 567–570.
226. Haworth, W. N.; Jones, W. G. M. *J. Chem. Soc.* **1944**, 667–670.
227. Antal, M. J.; Mok, W. S. L.; Richards, G. N. *Carbohydr. Res.* **1990**, *199*, 91–109.
228. Kuster, B. M. F. *Starch/Stärke* **1990**, *42*, 314–321.
229. Harris, J. F.; Saeman, J. F.; Zoch, L. L. *For. Prods J.* **1960**, *10*, 125–128.
230. Montgomery, R.; Wiggins, L. F. *J. Soc. Chem. Ind.* **1947**, *66*, 31–32.

Downloaded by DUKE UNIV on June 17, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch009

231. Middendrop, J. A. *Rec. Trav. Chim.* **1919**, *38*, 1–71.
232. Kuster, B. F.; van der Baan, M. H. S. *Carbohydr. Res.* **1977**, *54*, 165–176.
233. Kuster, B. F. M. *Carbohydr. Res.* **1977**, *54*, 177–183.
234. Kuster, B. F. M.; Temmink, H. M. G. *Carbohydr. Res.* **1977**, *54*, 185–191.
235. Moye, C. J.; Krzeminski, Z. S. *Aust. J. Chem.* **1963**, *16*, 258–269.
236. Hamada, K.; Yoshihara, H.; Suzukamo, G. *Chem. Lett.* **1982**, 617–618.
237. Fayet, C.; Gelas, J. *Carbohydr. Res.* **1983**, *122*, 59–68.
238. Schraufnagel, R. A.; Rase, H. F. *Ind. Eng. Chem. Prod. Res. Dev.* **1975**, *14*, 40–44.
239. El Hajj, T.; Masroua, A.; Martin, J. C.; Descotes, G. *Bull. Soc. Chim. Fr.* **1987**, 855–860.
240. Masroua, A.; Revillon, A.; Martin, J. C.; Guyot, A.; Descotes, G. *Bull. Soc. Chim. Fr.* **1988**, 561–566.
241. Vinke, P.; van Bekkum, H. *Starch/Starke* **1992**, *44*, 90–96.
242. Rigal, L.; Gaset, A.; Gorrichon, J. P. *Ind. Eng. Chem. Prod. Res. Dev.* **1981**, *20*, 719–721.
243. Rigal, L.; Gorrichon, J. P.; Gaset, A.; Heughebaert, J. C. *Biomass* **1985**, *7*, 27–45.
244. Rigal, L.; Gaset, A. *Biomass* **1985**, *7*, 267–276.
245. Jow, J.; Rorrer, G. L.; Hawley, M. C. *Biomass* **1987**, *14*, 185–194.
246. Moreau, C.; Durand, R.; Pourcheron, C.; Razigade, S. *Ind.Crops Prod.* **1994**, *3*, 85–90.
247. Rivalier, P.; Duhamet, J.; Moreau, C.; Durand, R. *Catal. Today* **1995**, *24*, 165–171.
248. Rapp, M. K. E.P. Patent 0,230,250, 1987.
249. Bonner, T. G.; Bourne, E. J.; Ruszkiewicz, M. *J. Chem. Soc.* **1960**, 787–791.
250. Brown, D. W.; Floyd, A. J.; Kinsmann, R. G.; Roshan-Ali, Y. *J. Chem. Technol. Biotechnol.* **1982**, *32*, 920–924.
251. Musau, R. M.; Munavu, R. M. *Biomass* **1987**, *13*, 67–74.
252. Nakamura, Y.; Morikawa, S. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 3705–3706.
253. Carlinia, C.; Giuttaria, M.; Gallettia, A. M. R.; Sbranaa, G.; Armarolib, T.; Buscab, G. *Appl. Catal., A* **1999**, *183*, 295–302.
254. Peniston, Q. P. U.S. Patent 2,750,394, 1956.
255. Hales, R. A.; Le Maistre, J. W.; Orth, G. O. U.S. Patent 3,071,599, 1963.
256. Kuster, B. F. M.; Laurens, J. *Starch/Starke* **1977**, *29*, 172–176.
257. Chheda, J. N.; Roman-Leshkov, Y.; Dumesic, J. A. *Green Chem.* **2007**, *9*, 342–350.
258. Roman-Leshkov, Y.; Barrett, C. J.; Liu, Z. Y.; Dumesic, J. A. *Nature* **2007**, *447*, 982–985.
259. Roman-Leshkov, Y.; Chheda, J. N.; Dumesic, J. A. *Science* **2006**, *312*, 1933–1937.
260. Binder, J. B.; Raines, R. T. *J. Am. Chem. Soc.* **2009**, *131*, 1979–1985.
261. Zhang, Z. C. *Adv. Catal.* **2006**, *59*, 153–237.
262. Zhao, H. B.; Holladay, J. E.; Brown, H.; Zhang, Z. C. *Science* **2007**, *316*, 1597–1600.
263. Yong, G.; Zhang, Y. G.; Ying, J. Y. *Angew. Chem., Int. Ed.* **2008**, *47*, 9345–9348.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch009

264. Mascal, M.; Nikitin, E. B. *Angew. Chem., Int. Ed.* **2008**, *47*, 7924–7926.
265. Qi, X. H.; Watanabe, M.; Aida, T. M.; Smith, R. L. *Catal. Commun.* **2008**, *9*, 2244–2249.
266. Bicker, M.; Kaiser, D.; Ott, L.; Vogel, H. *J. Supercrit. Fluids* **2005**, *36*, 118–126.
267. Gandini, A.; Belgacem, M. N. *Prog. Polym. Sci.* **1997**, *22*, 1203–1379.
268. Gandini, A.; Belgacem, N. M. *Polym. Int.* **1998**, *47*, 267–276.
269. Dykstra, C. C.; Boykin, D.; Tidwell, R. R. WO Patent 9,838,170, 1998.
270. Gauthier, D. R.; Szumigala, R. H.; Dormer, P. G.; Armstrong, J. D.; Volante, R. P.; Reider, P. J. *Org. Lett.* **2002**, *4*, 375–378.
271. Hopkins, K. T.; Wilson, W. D.; Bender, B. C.; McCurdy, D. R.; Hall, J. E.; Tidwell, R. R.; Kumar, A.; Bajic, M.; Boykin, D. W. *J .Med. Chem.* **1998**, *41*, 3872–3878.
272. Kobayashi, H.; Hagiwara, M. JP Patent 05,273,718, 1993.
273. Anderson, H. C. U.S. Patent 4,320,043, 1982.
274. Matsumoto, I.; Nakagawa, K.; Horiuchi, K. *Jpn. Kokai* **1973**, *73*, 763.
275. Timko, J. M.; Cram, D. J. *J. Am. Chem. Soc.* **1974**, *96*, 7159–7160.
276. Durant-Pinchard, M. FR Patent 2,556,344, 1985.
277. Gandini, A. In *Agricultural and Synthetic Polymers*; ACS Symposium Series 433; American Chemical Society: Washington, DC,1990; pp 195−208.
278. Koch, H.; Krause, F.; Steffan, R.; Woelk, H. U. *Starke* **1983**, *35*, 304–313.
279. Huber, G. W.; Chheda, J. N.; Barrett, C. J.; Dumesic, J. A. *Science* **2005**, *308*, 1446–1450.
280. Sasaki, M.; Adschiri, T.; Arai, K. *J. Agric. Food Chem.* **2003**, *51*, 5376–5381.
281. Timokhin, B. V.; Baransky, V. A.; Eliseeva, G. D. *Russ. Chem. Rev.* **1999**, *68*, 73–84.
282. Bozell, J. J.; Moens, L.; Elliott, D. C. *Resour. Conserv. Recy.* **2000**, *28*, 227–239.
283. Leonard, R. H. *Ind. Eng. Chem.* **1956**, *48*, 1331–1341.
284. Kitano, M.; Tanimoto, F.; Okabayashi, M. *Chem. Econ. Eng. Rev.* **1975**, *7*, 25–29.
285. Mascal, M.; Nikitin, E. B. *Green Chem.* **2010**, *12*, 370–373.
286. Ghorpade, V. Hanna, M. A. In *Cereal Novel Uses and Processes*; Campbell, G. M., Webb, C., McKee, S. L., Eds.; Plenum Press: New York, 1997; pp 49–55.
287. Mulder, G. J. *J. Prakt. Chem.* **1840**, *21*, 219.
288. Timokhin, B. V.; Baransky, V. A.; Eliseeva, G. D. *Russ. Chem. Rev.* **1999**, *68*, 80–93.
289. Girisuta, B. Ph.D. Thesis, University of Groningen, Groningen, Netherlands, 2007.
290. Harris, D. W.; Feather, M. S. *J. Am. Chem. Soc.* **1975**, *97*, 178–181.
291. Horvat, J.; Klaic, B.; Metelko, B.; Sunjic, V. *Tetrahedron Lett.* **1985**, *26*, 2111–2114.
292. Itaya, H.; Shiotani, A.; Toriyahara, Y. JP Patent 62,252,742, 1998.
293. Otsuka, M.; Hirose, Y.; Kinoshita, T.; Masawa, T. U.S. Patent 3,752,849, 1973.

Downloaded by DUKE UNIV on June 17, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch009

294. Farnleitner, L.; Stueckler, H.; Kaiser, H.; Kloimstein, E. German Patent 3,920,340, 1991.
295. Vaerman, J. M.; Bertrand, J. N. M. German Patent 2,125,162, 1972.
296. Fuentes, L. M.; Larson, G. L. *Tetrahedron Lett.* **1982**, *23*, 271–274.
297. Edwards, W. B. U.S. Patent 4,612,391, 1986.
298. Cavinato, G.; Toniolo, L. *J. Mol. Catal.* **1990**, *58*, 251–267.
299. Ballini, R.; Petrini, M. *Synthesis* **1986**, 1024–1026.
300. Fucutes, L.; Larson, G. *Tetrahedron Lett.* **1982**, *23*, 271.
301. Baugh, K. D.; McCarty, P. L. *Biotechnol. Bioeng.* **1988**, *31*, 50–61.
302. Qi, F.; Hanna, M. A. *Bioresour. Technol.* **2002**, *81*, 187–192.
303. Fitzpatrick, S. W. U.S. Patent 5,608,105, 1995.
304. Cha, J. Y.; Hanna, M. A. *Ind. Crops Prod.* **2002**, *16*, 109–118.
305. Farone, W. A.; Cuzens, J. U.S. Patent 6,054,611, 2000.
306. Chang, C.; Cen, P.; Ma, X. J. *Bioresour. Technol.* **2007**, *98*, 1448–1453.
307. Bart, H. J.; Reidetschlager, J.; Schatka, K.; Lehmenn, A. *Ind. Eng. Chem. Res.* **1994**, *31*, 21–25.
308. Olah, G. A.; Welch, J. T. *Synthesis* **1974**, 652.
309. Khim, Z. JP Patent 4,327, 1956.
310. Bacler, A. R.; Kontowicz, A. B. *J. Am. Chem. Soc.* **1953**, *75*, 118.
311. Khim, Z. Aust. Patent 277,666, 1969.
312. Kotrelev, V. N.; Rubtsova, I. K. *Khim. Promst.* **1953**, *8*, 46.
313. Drefahl, G.; Gros, B. *J. Prakt. Chem.* **1955**, *1*, 153.
314. Seigo, K.; Usui, M.; Kikuchi, K. *Bull. Chem. Soc. Jpn.* **1977**, *51*, 1863.
315. He, Z.; Zhao, L. *Huaxue Yanjiu Yu Yingyong* **2001**, *13*, 537–548.
316. *Ethyl Levulinate D-975 Diesel Additive Test Program*; Texaco/NYSERDA/Biofine, Inc.: Glenham, NY, 2000.
317. Manzer, L. E.; Herkes, F. E. U.S. Patent 0,192,933 A1, 2004.
318. Isoda, Y.; Azuma, M. JP Patent 08,053,390, 1996.
319. Hoogeboom, T. J. U.S. Patent 3,816,373, 1974.
320. Demmer, C. G.; Irving, E. EP Patent 184,553, 1986.
321. Chu, F.; Hawker, C. J.; Pomery, P. J.; Hill, D. J. T. *J. Polym. Sci., Part A: Polym. Chem.* **1997**, *35*, 1627–1633.
322. Weber, M.; Weiser-Elbl, K. EP Patent 855,430, 1998.
323. Chafetz, H.; Liu, C. S.; Papke, B. L.; Kennedy, T. A. U.S. Patent 5,445,750, 1995
324. Rosenquist, N. EP Patent 372,323, 1991.
325. Holmen, R. E.; Olander, S. J. U.S. Patent 4,031,048, 1977.
326. Ha, H. J.; Lee, S. K.; Ha, Y. J.; Park, J. W. *Synth. Commun.* **1994**, *24*, 2257–2562.
327. Benedikt, E.; Kost, H. P. Z. *Z. Naturforsch., B: Anorg. Chem., Org. Chem.,Biochem., Biophys., Biol.* **1986**, *41*, 1593–1594.
328. Rebeiz, N.; Arkins, S.; Rebeiz, C. A.; Simon, J.; Zachary, J. F.; Kelley, K. W. *Cancer Res.* **1996**, *56*, 339–344.
329. Appleton, D; Duguid, A. B; Lee, S. K.; Ha, Y. L.; Ha, H. J.; Leeper, F. J. J. *Chem. Soc., Perkin Trans.* **1998**, *1*, 89–101.
330. Schuette, H. A.; Thomas, R. W. *J. Am. Chem. Soc.* **1930**, *52*, 3010–3012.
331. Kyrides, L. P.; Groves, W.; Craver, J. K. U.S. Patent 2,368,366, 1945.

Downloaded by DUKE UNIV on June 17, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch009

332. Christian, R. V. J.; Brown, H. D.; Hixon, R. M. *J. Am. Chem. Soc.* **1947**, *69*, 1961–1963.
333. Broadbent, H. S.; Campbell, G. C.; Bartley, W. J.; Johnson, J. H. *J. Org. Chem.* **1959**, *24*, 1847–1854.
334. Joo, F.; Somsak, L.; Beck, M. T. *J. Mol. Catal.* **1984**, *24*, 71–75.
335. Joo, F.; Toth, Z.; Beck, M. T. *Inorg. Chim. Acta* **1977**, *25*, L61–L62.
336. Osakada, K.; Ikariya, T.; Yoshikawa, A. *J. Organomet. Chem.* **1982**, *231*, 79–90.
337. Bracca, G.; Raspolli-Galletti, A. M.; Sbrana, G. *J. Organomet. Chem.* **1991**, *417*, 41–49.
338. Aminoff, C.; Vanninen, E.; Doty, T. E. In *Xylitol*; Counsell, J. N., Ed.; Applied Science Publishers: London, 1978.
339. Nikolaev, D. I.; Chernikova, L. P.; Glazman, B. A.; Kostyuk, I. N.; Rutskaya, M. S.; Chivyaga, A. A. *Gidroliz. Lesokhim. Prom-St.* **1983**, *2*, 16–18.
340. Kind, V. B.; Vyglazov, V. V.; Kholkin, Y. J. *Gidroliz. Lesokhim. Prom-St.* **1987**, *3*, 11–12.
341. Wisniak, J.; Hershkowitz, M.; Leibowitz, R.; Stein, S. *Ind. Eng. Chem. Prod. Res. Develop.* **1974**, *13*, 75–79.
342. Mikkola, J. P.; Salmi, T.; SjoÈholm, R. *J. Chem. Technol. Biotechnol.* **1999**, *74*, 655–662.

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Chapter 10

Chemicals from Lignin Based on Thermal Fusibility and Amphiphilicity

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Development of low-cost, value-added bio-materials from technical lignins is essential in terms of complete utilization of woody biomass components (biorefining). A simply designed chemical modification may provide technical lignins with some unique properties: thermal fusibility, thermal moldability, amphiphilicity, and miscibility with synthetic polymers. Here, the fundamental characteristics of technical lignins are first overviewed. Second, recent progress in chemical modification of technical lignins for industrial application is reviewed. Finally, newly developed lignin-containing materials with functionality are discussed.

Introduction

Utilization of biomass resources in place of fossil fuels has gained a great deal of attention owing to the recent surge in environmental consciousness (*1*). Woody biomass is an important source of biomass as a result of its abundance. Indeed, it has been estimated that forests contain up to 80% of all above-ground carbon on earth (*2*). Attempts to utilize all of the components of woody biomass in the form of chemicals and energy are commonly referred to as biorefining (*3*). Lignin, which is one of the major components of wood cell walls, is believed to be the most abundant natural aromatic polymer on the planet (*4*). Accordingly, research to develop lignin-based materials and/or polymers for their industrial application have been ongoing for decades.

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However, development of lignin-based materials is essentially a challenge for several reasons. Unlike other natural polymers such as cellulose, starch, protein and nucleotide, lignin is very complex and contains irregular interunitary linkages that each have different frequencies. This complexity is a result of the resonance-stabilized coupling processes, which involve phenoxy radicals that occur during lignin biosynthesis (*5*). This constitutional fluctuation of lignin is a drawback and is one of characteristics that makes it unsuitable for application as an industrial feedstock. Moreover, to enable their widespread use, lignin-based materials should possess physical and/or chemical properties that are at least comparable to those of petroleum-based materials. However, the inhomogeneity of the lignin structure (*6*, *7*) is often an obstacle against production of lignin-based materials of acceptable, regular quality. In addition, lignin contains only a small number of reactive sites, which limits the ability of the polymerization process to fabricate lignin-derived polymers such as lignin-based phenol-formaldehyde resin (*8*). Finally, for a novel, profitable lignin-based biomaterial developed by chemical modification of kraft lignin to be useful for industrial applications, it is necessary for the process used to be more cost-effective than energy production from thermal recycling of the lignin during the kraft pulping process. This is because the kraft pulping process is a well-organized, capital-intensive system that effectively recycles chemicals and energy.

Nevertheless, newly-developed processes such as organosolv pulping (*9*) and steam explosion (*10*) have led to the development of a variety of techniques for the preparation of lignin with unique properties, stimulating research and development of lignin-based materials.

This study overviews the basic properties of technical lignins and their derivatives first, and then, introduces recent progress in the development of lignin-based biomaterials for their industrial application.

1. Types of Technical Lignin and Chemical Modification for Their Application

Many studies have been conducted to develop separation methods for the isolation of lignins from plant materials such as wood, grass, and crop residues (*11*, *12*). Additionally, various lignins have been obtained as byproducts of industrial processes, in which carbohydrates (mainly cellulose) were used as the main products. Two types of methods are commonly used to isolate lignin from plant materials. In one, the lignin is selectively solubilized in pulping solvent, after which it is collected as precipitate in poor solvent. In the kraft pulping process, a lignin-rich fraction containing chemically modified lignin is obtained from pulping processes as a water-soluble fraction, primarily under alkali conditions, while carbohydrates are separated as a water-insoluble fraction. Lignin preparation can be recovered by precipitating the lignin from the water-soluble fraction under acidic conditions. In the other method, carbohydrates are selectively solubilized in a solvent system in which lignin remains as an insoluble fraction. This method is typically employed in bioethanol production (*13*) using carbohydrate-degrading enzymes and organic or inorganic acids, which enables lignin to be obtained as a water-insoluble fraction whereas carbohydrates are separated as a water-soluble fraction. However, it is quite difficult to separate lignin from plant materials without structural modification, regardless of which method is used (*14*, *15*). Chemical modification of a lignin macromolecule significantly influences its chemical and physical properties (*16*). Therefore, the chemical and physical properties of a lignin preparation are highly dependent on the conditions used for its separation.

Some typical methods used to convert technical lignins to lignin-based biopolymers are addressed below. Emphasis is placed on the synthesis of thermosetting polymers such as lignin-based phenol-formaldehyde resin and epoxy resin, where polymerization reactions are based on the reactivity of the phenolic and aliphatic hydroxyl groups and the nucleophilic nature of the phenol that lignin possesses.

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1.1. Lignosulfonate

Lignosulfonate (sulfite lignin; LS) is produced as a byproduct during the sulfite pulping processes, which heralded the dawn of modern pulping technology. The pH profile used for the pulping processes is a crucial factor influencing the chemical properties of LS (*17*). All types of LS are highly hydrophilic owing to the presence of sulfonyl groups, which have strong polarity and acidity, introduced during the pulping processes. Therefore, LS shows amphiphilicity and is used commercially as a surfactant. Low-cost surfactants that can be adsorbed onto cement particles at very low concentrations are of great interest on a commercial basis (*18*). Indeed, such surfactants have been widely used as cement dispersants or water-reducing admixtures of concrete (*19*, *20*).

LS has been studied as a raw material for *lignin-based resin* for some historical reasons by developing and emerging capital-intensive technologies and systems in modern pulp and paper industries. However, LS has some disadvantages for use in the production of waterproof resin. Specifically, it was reported that extension of the curing time and temperature to hydrolyze sulfonyl groups from resin could improve the waterproofing properties of lignosulfonate-formaldehyde resin (*21*). The major thermal decomposition of LS generally starts at 250-300 ºC. However, minor decomposition of LS was also observed below 200 ºC (*22*). Accordingly, a high curing temperature would be a potential problem in the production and thermal stability of lignin-based composite materials. Sulfonyl group content can also be reduced by chemical modification. By heating LS in phenols, phenolation occurs at the benzylic position, eliminating sulfonyl groups (*23*). Lignin generally shows poorer reactivity with formaldehyde in condensation reactions than phenol does, because the methoxyl groups of lignin are located and blocked at the ortho position to its free phenolic hydroxyl groups. Thus, phenolation increases the number of reactive sites in lignin preparations. In a much simpler method, lignin is simply mixed with phenol. In this process, as much as 40-60% of phenol can be replaced by lignin (*24*). However, the relatively low amount of hydroxyl groups in LS (*25*) would reduce its usefulness for the synthesis of other types of lignin-based polymer, such as polyurethane and epoxy resins.

1.2. Kraft Lignin

Kraft pulping processes were later developed to improve the strength and/or the stiffness of the pulp and the resulting paper. Kraft pulping processes have recently replaced sulfite processes as the leading technology for pulping worldwide.Therefore, kraft lignin (sulfate lignin; KL) is one of the most available technical lignins produced by the modern pulp and paper industries. During the kraft pulping processes, almost all of the aryl-ether linkages in lignin macromolecules are cleaved (*26*). As a result, KL has a high phenolic hydroxyl group content, exhibiting relatively high reactivity with electrophiles such as isocyanate and epoxy compounds to fabricate resins, when compared to LS. The molecular weight of KL is much lower than that of LS, which is an advantage in reactivity. Nevertheless, its reactivity is limited when compared to that of low molecular phenols. Thus, chemical modification of KL has been attempted to overcome this limited reactivity and enable fabrication of lignin-based polymers so that they are incorporated as the skeleton of the polymers. Hydroxyalkylation (*27–29*), epoxylation (*30*), and isocyanation (*31*, *32*) are representative examples.

Once introduced, KL would likely act as a hard segment in these resins (*33*). Therefore, introduction of KL into the resins can be expected to improve their mechanical properties such as stiffness and/or strength. KL-phenol-formaldehyde resin (KLPF resin) was investigated in terms of its application as a binder for plywood (*34*, *35*), oriented strandboard (*36*), flakeboard (*37*), and particleboard (*38*). KLPF resin with about 50% substitution of KL for phenol was found to contribute better mechanical properties to these wood-based panels.

Softwood KL (including its phenolated derivatives) based epoxy resins show high adhesition to aluminum (> 70 kg/cm^2) and wood (> 60 kg/cm^2) (*39*). The adhesive shear strength of epoxy resins can also be dependent on the lignin content (*40*). Addition of KL to a commercial epoxy resin was found to increase the adhesive joint shear strength with the lignin content up to 35% and it reached its maximum value (> 170% of that of commercial resin) when 20% of lignin content was applied (*40*, *41*). The mechanical properties of KL-epoxy resin strongly depend on the molecular structure of the glycidylether used, and thus can be tailor-made (*42*)

The thermal properties of a lignin-based epoxy resin are also affected by the lignin content and the chemical structure of glycidylether. Introduction of KL to the resin brings about a shift in glass transition temperature (Tg) to higher values (*43*). This can be explained by the formation of a chemical bond between a lignin molecule (with high Tg) and the epoxy resin. However, some of the lignin domain can remain immiscible with the epoxide network, which may cause variations in the mechanical properties of the epoxy resin. Controlling the concentration of a curing agent can be a solution to this problem (*44*). In the case of lignin-epoxy resin from polyethylene glycol diglycidyl ethers, DMA (dynamic mechanical analysis) revealed that the lignin domain exists in a well-miscible manner in lignin-epoxy resin (*42*). These differences may be a result of the reactivity of lignin molecules in different epoxy systems, or the high miscibility of the lignin with ethylene oxide groups (*45*). Lignin-based epoxy resins have already been patented (*46*).

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There are also some patents on KL-based polyurethane resins. Mechanical properties and nonflammability have been discussed for kraft lignin- (including its carboxylated derivatives) based polyurethane (*47*).

1.3. Other Technical Lignin Preparations

It has been reported that sulfur-free organosolv lignins have relatively lower amounts of phenolic hydroxyl groups (*48*, *49*), higher molecular mass (*48*), and lower T_g values (*49*) than those of KL and LS. As discussed later, the relatively low T_g value of organosolv lignins results in their being more easily thermally molded than kraft lignins (*50*), which has led to a new method for the development of organosolv lignin-based thermoplastic resin.

In terms of the development of thermosetting resin, there have been several studies of organosolv lignin-based epoxy and polyurethane resins. The mechanical properties of polyurethane resin can also be improved by the addition to Alcell lignin (*51*). The glass transition temperature of Alcell lignin-based epoxy resin was found to increase with increasing lignin content (*52*), as observed in kraft lignin-based epoxy resin (*43*). When Alcell lignin and its carboxylated derivatives were pretreated chemically before being introduced to epoxy resin, a shift in T_g of the resulting resin to a higher value was observed, as in kraft lignin and its derivatives (*53*).

Steam exploded lignin has been discussed as a promising raw material for epoxy resin production (*54*). Methanol soluble lignin separated from hardwood by steam explosion was used to prepare lignin-based epoxy resin (*55*). This methanol soluble fraction has a relatively high phenolic hydroxyl content with a low molecular mass range (Mw = 800-1,000). As a result, the curing time of the lignin-based epoxy resin was reduced to one-fifth of that of bisphenol A-based epoxy resin (*55*).

2. Thermal Fusibility and Moldability of Lignin-Based Materials

One of the most remarkable features of synthetic polymers is their thermal moldability, which is based on their thermal fusibility and enables their easy transformation into fibers, films, and moldings. The thermal fusibility of a polymer is considered an essential factor that is closely related to thermally-assisted extrusion moldability and injection moldability (*56*, *57*). Obviously, it is desirable to easily mold lignin-based polymers into their intended form. Application of industrial lignins would likely expand if they are provided with thermal fusibility. However, the lignin-based polymers discussed above have almost no thermal fusibility, much less thermal moldability, because they are essentially thermosetting resins. Accordingly, a great deal of efforts has been made to produce lignin-based materials with thermal fusibility or moldability (*58*). The fact that a simple chemical modification can turn technical lignin into thermal fusible materials has led to development of novel methods of utilizing lignins to produce carbon fibers (*59*, *60*) and hot-melt adhesives (*61*). The development of

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
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lignin-based polymers prepared by chemical modification on the basis of thermal moldability is described below.

2.1. Thermal Fusibility of Lignin Derivatives

If a polymer shows thermal fusibility, it can be transformed into thermal moldings such as fibers and film. In addition to T_g, the thermal-flow starting temperature, which corresponds to the melting temperature (T_m) of crystalline polymers, is an important indicator that can be used to estimate the thermal fusibility of an amorphous polymer.

Until the middle of the 1990s, isolated lignins were believed to be non-fusible material upon heating. Glasser et al. made attempts to develop fusible material by chemical modification of isolated lignins (*62*). Glasser and Jain found that lignin alkanoates, obtained by acylation of non-sulfonated lignins with aliphatic monocarboxylic anhydrides and pyridine, exhibited distinctly discerning T_g with the ability to undergo thermal flow (*62*). They also reported that the lignin alkanoates having monocarboxylic moieties with longer alkyl chains showed lower thermal softening temperatures (*62*).

Later, Sudo et al. developed two new methods of preparing fusible lignin derivatives from chemically modified steam-exploded birch lignins. One was hydrogenolysis to convert it into a thermally fusible material (*63*). The other was phenolation, which lowered the T_g value of the steam exploded lignin, and allowed it to easily enter a molten state (*64*). The phenolated lignin, showing good spinnability, was successfully converted to carbon fibers with a fairly high yield (over 40%) (*64*).

2.2. Thermal Fusibility of Isolated Lignins and Their Application

It is possible to reduce the costs of fabricating lignin-based carbon fibers, if isolated lignins exhibit thermal fusibility without derivatization. Uraki et al. found that birch lignin obtained from an aqueous acetic acid pulping process under atmospheric pressure in an organosolv pulping process had thermal fusibility upon heating (*59*). They also successfully prepared lignin fibers from the hardwood lignin by melt spinning (*59*). Kubo et al. later found that thermomechanical analysis (TMA) was the best way to characterize the thermal behavior, such as the T_g and thermal-flow starting temperature, of powdered lignins (*65*). DSC is generally and widely used for thermal analysis, but this technique has been found to be inappropriate for monitoring of the thermal fusibility of amorphous polymers such as lignin preparations and lignin-based polymers (*65*). DSC cannot detect a clear phase transition because thermal flow is a continuous process that leads to the initiation of macro-Brownian motion of polymer chains. They demonstrated the effect of the occurrence and the frequency of the condensed structure of lignin on its thermal mobility using TMA (*65*). The principle of TMA is illustrated in Figure 1, which shows clear profiles for three types of technical lignin, HAL (hardwood acetic acid lignin; fusible), SKL (softwood kraft lignin; infusible), and EL (steam exploded lignin; infusible). The TMA profile of HAL (Route A) provided two inflection points. The profile of SKL (Route B) gave only one inflection point.

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The TMA profile of EL exhibited a gradual decrease in volume up to a certain temperature, and then showed volume expansion at higher temperatures , similar to SKL (Route B), followed by a drastic decrease in volume (Route C).

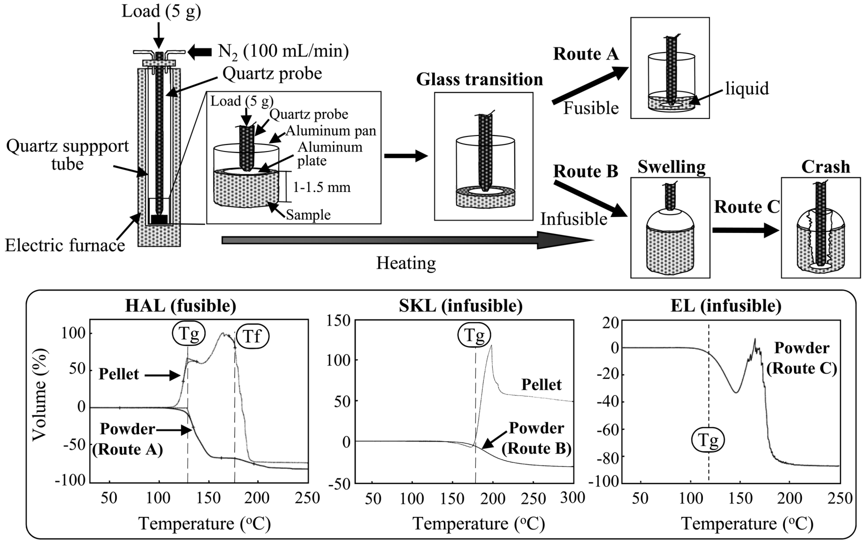
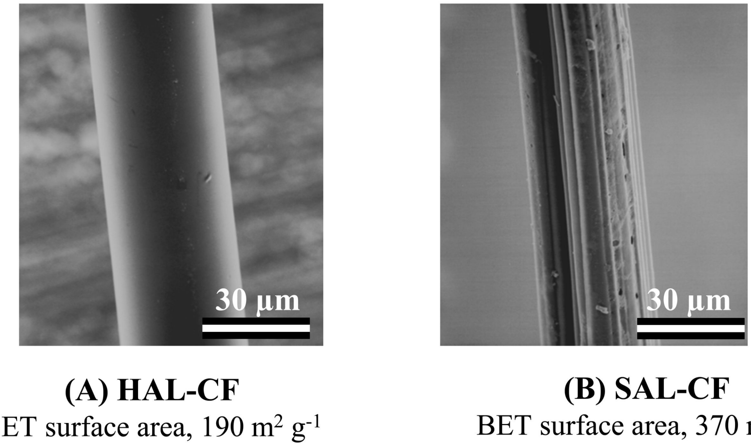


Figure 1. Apparatus for TMA (top) and illustration of three types TMA profile of technical lignins: hardwood acetic acid lignin [HAL] type (bottom left), softwood kraft lignin [SKL] type (bottom middle), and hardwood steam-exploded lignin [EL] type (bottom right).

TMA revealed that HAL had a glass transition temperature at 128 ºC, which is shown as the first inflection point and represents the reduction in the void volume of the test sample as air escaped from the airspace among lignin particles under the 5 g loading of the probe. TMA also showed that HAL had a thermal-flow starting temperature at 177 ºC, which produced the second inflection point and represented lignin being in the liquid state with the probe in the sample (*65*). Thus, Kubo et al. confirmed that HAL showed thermal fusibility. HAL was successfully subjected to melt spinning after thermal treatment to remove the volatile components, after which it was further converted to carbon fibers by carbonization at 1000 ºC (Figure 2-A; HAL-CF) (*65*). The thermal fusibility of HAL was also confirmed to be caused by the acetyl group introduced during the acetic acid pulping process as an internal plasticizer, and by the low-molecular mass fraction of the HAL as an external plasticizer (*65*). Conversely, SAL did not exhibit thermal fusibility (*60*). These findings can partially be explained by the nature of softwood lignin. Specifically, this forms of lignin is rich in guaiacyl-type condensed structure bonded at position 5 of the aromatic nuclei, which likely limits the rotational mobility of the aromatic skeleton of lignin (*60*). However, infusible SAL was successfully converted into a fusible material by removing the infusible, high-molecular mass fraction, or by extensively cleaving the aryl-ether bond extensively by recooking the SAL (*60*). The obtained fusible lignin was spun by melt spinning, and the carbon fibers were prepared from the resulting fibers (Figure 2-B; SAL-CF) without thermostabilization (*66*).



(A) HAL-CF
BET surface area, 190 m^2 g^{-1}

(B) SAL-CF
BET surface area, 370 m^2 g^{-1}

Figure 2. Scanning electron micrographs of hardwood acetic acid lignin [HAL] and softwood acetic acid lignin [SAL]-derived carbon fibers [CF].

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Kadla et al. has published a series of papers pertaining to lignin-based biomaterials, based on the thermal fusiblity of lignin. They also successfully transformed Alcell lignin, a commercially available organosolv lignin isolated by acidic ethanolysis of hardwood, into filament form without further chemical modification to give a suitable precursor for the formation of carbon fibers (*67*). Moreover, they first produced carbon fibers from commercially available hardwood KL without chemical modification, by melt spinning and carbonization (*67*).

Uraki et al. attempted to provide additional functionality to lignin-based carbon fibers by converting carbon fibers into activated carbon fibers. They successfully prepared activated carbon fibers with a large specific surface area from SAL (*68*) as well as HAL (*69*). They even succeeded in catalytic graphitization of HAL, although the graphitization of lignin was generally thought to be quite difficult to conduct (*70*). They observed that the addition of 5 wt.% (on HAL) addition of nickel acetate did not compromise the thermal fusibility of HAL, and after thermal molding, highly crystalline carbon was produced from HAL directly.

2.3. Lignin/Synthetic Polymer Blends

Fibers from lignin and its derivatives produced by melt-spinning generally show poor mechanical properties, and have to be handled with care. Improvement of their mechanical properties is prerequisite for their industrial application. Kubo and Kadla investigated lignin-synthetic fiber composite using synthetic polymers/organosolv lignin blends in terms of their thermal properties, chemical structure, and blend behavior (*50*). Kadla and Kubo reported that in lignin-based polymer blends, poly(ethylene oxide) (PEO)/and poly(ethylene terephthalate) (PET)/lignin polymer blends were miscible, whereas poly(vinyl alcohol) (PVA)/and polypropylene (PP)/lignin polymer blends were immiscible (*71*).

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2.4. Application of Lignin-Based Amphiphilic Derivatives

Uraki et al. developed an amphiphilic lignin-hydrophilic polymer composite with the goal of fabricating a lignin-based functional surfactant that mimicked amphiphilic lignin-carbohydrate complex (LCC) occurring in living trees (*72*). They reported that amphiphilic lignin derivatives were successfully prepared by derivatizing some technical lignins such as acetic acid lignin (AL), kraft lignin (KL), and lignosulfonate (LS) with three types of PEG analogues containing an epoxy group; namely, polyethylene glycol diglycidylether (PEGDE; Figure 3-A), ethoxyl (2-hydroxy)propoxy polyethylene glycol diglycidylether (EPEG; Figure 3-B), and dodecyloxy- polyethylene glycol diglycidylether (DAEO; Figure 3-C) (*73*, *74*). They extensively investigated the surface activity of those lignin derivatives (Figure 4), in comparison with that of Triton X-100 (structure shown in Figure 3-D), a commercially available surfactant. They found that kraft lignin-DAEO derivatives exhibited the highest surface activity among those investigated, and thus the lowest critical micelle concentration (CMC), making it superior even to Triton X-100 (Figure 4). The results also revealed that DAEO reinforced the surface activity of LS (Figure 5). Moreover, the flow value of calcinated gypsum in the presence of LS/DAEO derivatives was confirmed to have higher dispersibility than that in the presence of LS (Figure 6). Therefore, amphiphilic lignin/DAEO derivatives are expected to be applied to cement dispersant (*74*). Their derivatives were also found to be useful as a cellulase-aid agent for improvement of cellulolytic saccharification of unbleached pulps, and enabling repeated use of cellulase in addition to maintenance of the activity of the enzyme in a buffer solution for a prolonged period of time (Figure 7) (*75*). These results were likely obtained because amphiphilic lignin moieties prevent cellulase from becoming irreversibly adsorbed onto the substrate or the residual lignin in the substrate (*76*).

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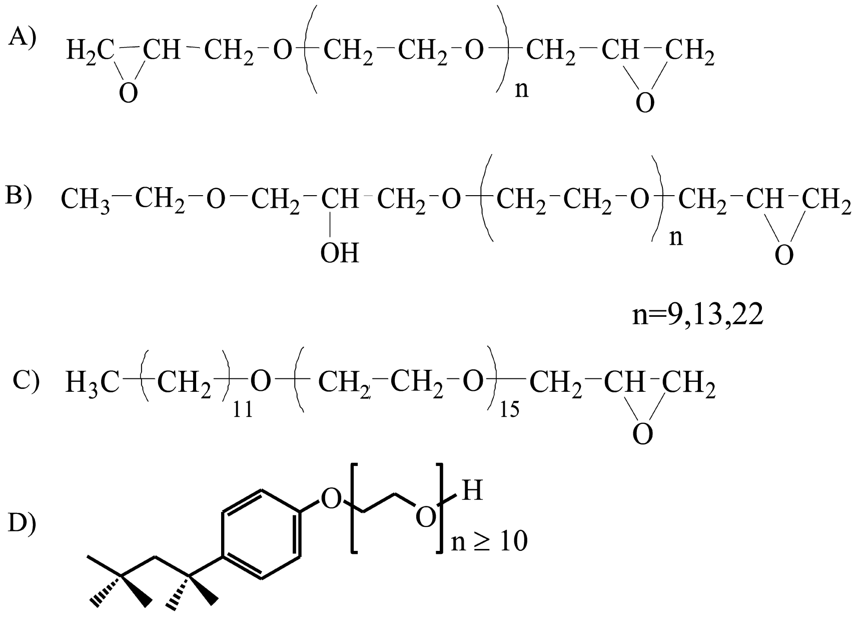

Figure 3. Chemical structure of polyethylene glycol diglycidylether (PEGDE, A), ethoxy (2-hydroxy)propoxy polyethylene glycol glycidylether (EPEG, B) and dodecyloxy-polyethylene glycol glycidyl ether (DAEO, C), and Triton X-100 (D).

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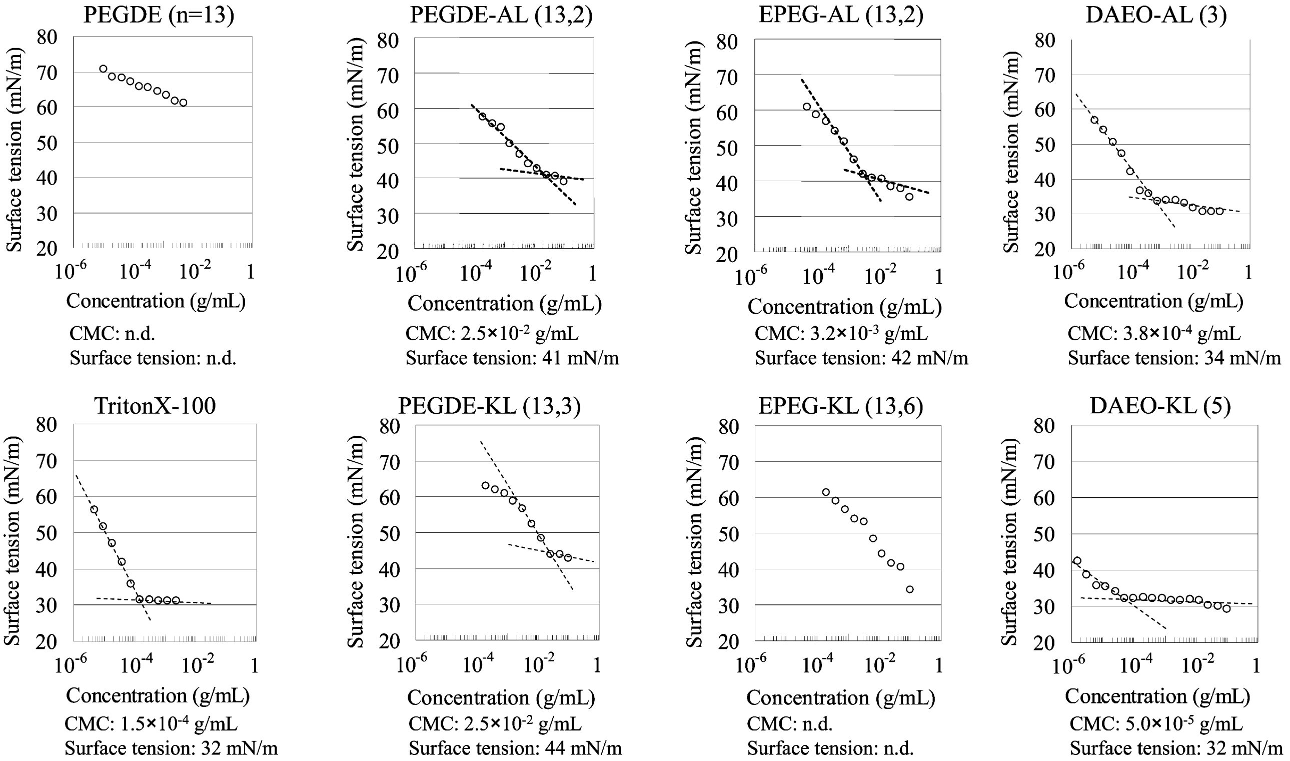

PEGDE (n=13): CMC: n.d.
Surface tension: n.d.

PEGDE-AL (13,2): CMC: 2.5×10^{-2} g/mL
Surface tension: 41 mN/m

EPEG-AL (13,2): CMC: 3.2×10^{-3} g/mL
Surface tension: 42 mN/m

DAEO-AL (3): CMC: 3.8×10^{-4} g/mL
Surface tension: 34 mN/m

TritonX-100: CMC: 1.5×10^{-4} g/mL
Surface tension: 32 mN/m

PEGDE-KL (13,3): CMC: 2.5×10^{-2} g/mL
Surface tension: 44 mN/m

EPEG-KL (13,6): CMC: n.d.
Surface tension: n.d.

DAEO-KL (5): CMC: 5.0×10^{-5} g/mL
Surface tension: 32 mN/m

Figure 4. Isotherms of surface tension of water-concentration of amphiphilic lignin derivatives. The abbreviations of PEGDE, EPEG and DAEO are referred to in Figure 3. Parentheses indicate the number of repeating unit of ethylene oxide residue in PEG moiety, and charge ratio of PEG derivatives with epoxy group to lignin (w/w).

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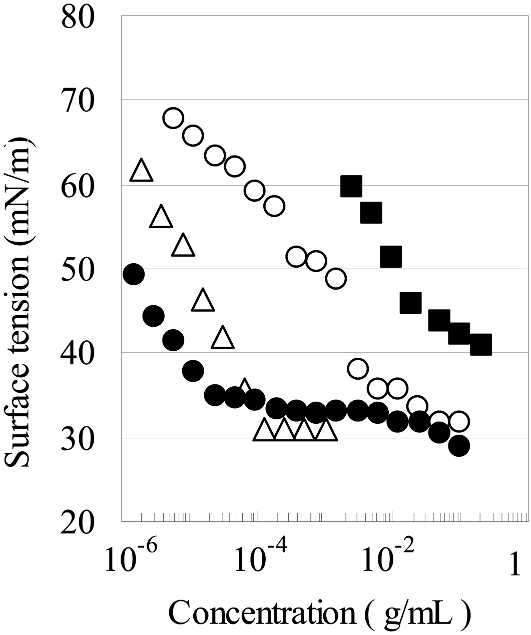

Figure 5. Surface tension-concentrations isotherms for LS, DAEO, and DAEO-LS. ■, LS; ○, DAEOLS(0.005); △, DAEO; ●, DAEO-LS (0.5).

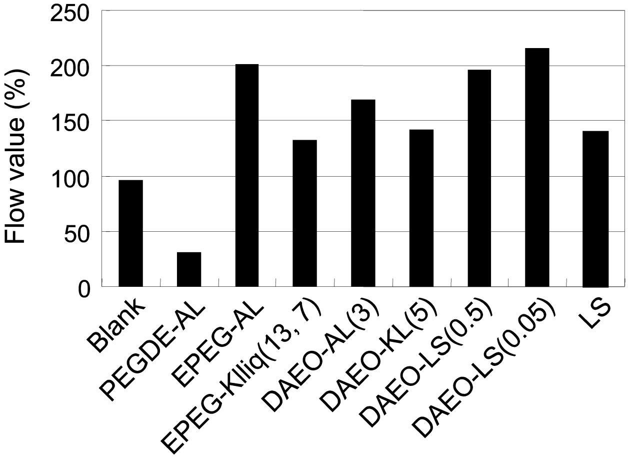

Figure 6. Flow values of caricinated gypsum in the presence of technical lignins and their derivatives.

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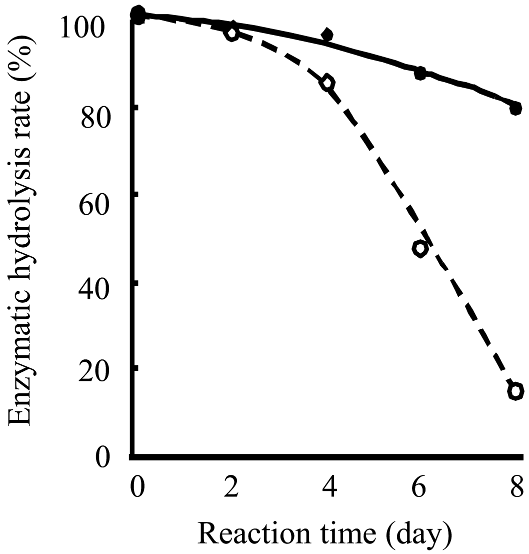

Figure 7. Decline in enzymatic hydrolysis rate of unbleached organosolv pulp by repeated use of cellulase. Hydrolysis conditions: Substrate, 3 g; Cellulase (Meicelase), 240 mg; PEGDE-AL, 0 wt.% (-○-) and 0.2 wt.% (—●—) based on 300 mL of the buffer solution.

2.5. Development of Novel Materials by the Use of Residual Lignin in Pulp

Uraki et al. focused on the hydrophobicity of residual lignin in unbleached pulp. An LCC-like, amphiphilic material was developed by derivatization of unbleached pulp to add water-solubility to its carbohydrate components, with the goal of utilizing the residual lignin in pulp, although this material was not prepared by the direct use of isolated lignins (*77*, *78*). Newly-developed, amphiphilic materials with functionality are introduced in this section.

Uraki et al. attempted to prepare lignocellulose-based, functional derivatives by hydroxypropylation of unbleached pulp, which formed self-aggregates in water (*79*). The presence of residual lignin in the derivatives was found to cause molecular association via hydrophobic interaction. This unique phenomenon was not observed in the corresponding cellulose derivative (hydroxypropyl cellulose; HPC) obtained from pure cellulose without lignin (*79*). The self-aggregate of the hydroxypropyl lignocellulosic derivatives adsorbed low molecular mass compounds to form inclusion compounds. The hydroxypropyl derivatives were also found to protect papain, a protease, against autolysis.

Uraki et al. further investigated the properties of the aqueous solution of hydroxypropyl lignocellulosics (*80*). Owing to the presence of residual lignin, the LCST (lowest critical solution temperature) of lignin-bearing HPC (HPC-L) in aqueous solution was observed at 38 ºC, which was lower than that of pure HPC (43 ºC). They attempted to develop a temperature-responsive, intelligent hydrogel from HPC-L. To accomplish this, they crosslinked HPC-L with PEGDE (n=1), which was later used to prepare lignin-based amphiphiles (*73*, *74*), and successfully prepared a hydrogel that showed a remarkable volumetric decrease at 38 ºC, as shown in Figure 8-A (*80*). The gel also repeatedly responded to the changes in ambient temperature (Figure 8-B). Therefore, Uraki et al. succeeded in the development of a body temperature-responsive, intelligent hydrogel from the lignocellulosic derivatives that could be applied to a polymer base drug delivery system (*80*).

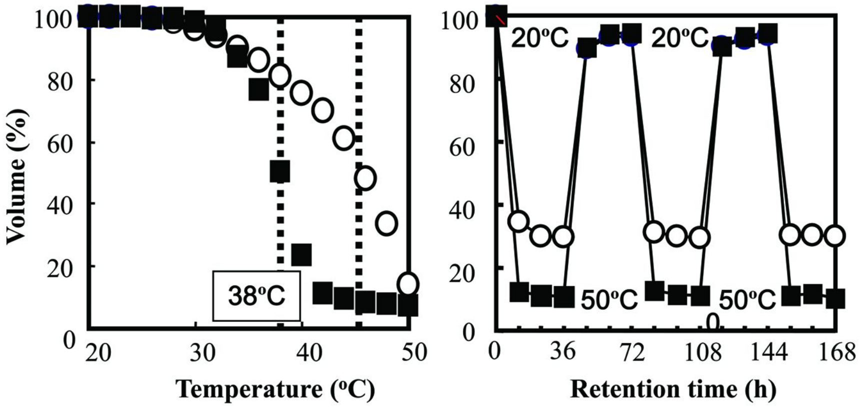

Figure 8. Temperature dependence of volume change of hydroxypropyl cellulose [HPC] gel (○) and lignin-bearing HPC [HPC-L] (■) gel. (A): stepwise heating at 2 ºC interval for 12 h from 20 ºC. (B): repeated heating between 20 ºC and 50 ºC.

As illustrated above, chemical modification or derivatization of lignin or lignocellulose can provide additional functionality to the original lignin or lignocellulose. Accordingly, it is expected that the development of lignin-based biopolymers carrying controlled functionality using carefully designed chemical modification processes will lead to their expanded application.

References

1. Shen, L.; Worrell, E.; Patel, M. *Biofuels, Bioprod. Biorefin.* **2010**, *4*, 25–40.
2. Dixon, R. K.; Brown, S.; Houghton, R. A.; Solomon, A. M.; Trexier, M. C.; Wisniewski *Science* **1994**, *263*, 185–191.
3. Amidon, T. E.; Liu, S. *Biotechnol. Adv.* **2009**, *27*, 542–550.
4. Lora, J. H.; Glasser, W. G. *J. Polym. Environ.* **1997**, *10*, 39–48.
5. Ralph, J.; Lundquist, K.; Brunow, G.; Lu, F.; Kim, H.; Schatz, P. F.; Marita, J. M.; Hatfield, R. D.; Ralph, S. A.; Christensen, J. H.; Boerjan, W. *Phytochem. Rev.* **2004**, *3*, 29–60.
6. Oennerud, H.; Gellerstedt, G. *Holzforschung* **2003**, *57*, 165–170.
7. Oennerud, H.; Gellerstedt, G. *Holzforschung* **2003**, *57*, 255–265.
8. El Mansouri, N. -E.; Salvado, J. *Ind. Crops Prod.* **2006**, *24*, 8–16.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch010

9. Nakamura, K.; Mörck, R.; Reimann, A.; Kringstad, K. P.; Hatakeyama, H. *Polym. Adv. Technol.* **1991**, *2*, 41–47.
10. Shimizu, K.; Sudo, K.; Ono, H.; Ishihara, M.; Fujii, T.; Hishiyama, S. *Biomass Bioenergy* **1998**, *14*, 195–203.
11. Janshekar, H.; Fiechter, A. In *Advances in Biochemical Engineering/ Biotechnology*; Fiechter, A., Jeffries, T. W., Eds.; Springer-Verlag: Berlin, Germany, 1983; Vol. 27, pp 119−178.
12. Brodin, I.; Sjöholm, E.; Gellerstedt, G. *Holzforschung* **2009**, *63*, 290–297.
13. Sassner, P.; Mårtensson, C. G.; Galbe, M.; Zacchi, G. *Biores. Technol.* **2008**, *99*, 137–145.
14. Pouwels, A. D.; Tom, A.; Eijkei, G. B.; Boon, J. J. *J. Anal. Appl. Pyrolysis* **1997**, *11*, 417–436.
15. Sjöholm, E.; Gustaffson, K.; Colmsjö, A. *J. Liq. Chromatogr. Relat. Technol.* **1999**, *22*, 1663–1685.
16. Sun, R. C.; Tomkinson, J. *Sep. Purif. Technol.* **2001**, *24*, 529–539.
17. Northey, R. A. In *Emerging Technology for Materials and Chemicals from Biomass*; Rowell, R. M., Schultz, T. P., Narayan, R., Eds.; ACS Symposium Series 476; American Chemical Society: Washington, DC, 1990; Ch. 11, pp 146−175.
18. Petrie, E. M. *Ind. Eng. Chem. Prod. Res. Dev.* **1976**, *15*, 242–249.
19. Monosi, S.; Moriconi, G.; Pauri, M.; Collepardi, M. *Cem. Concr. Res.* **1983**, *13*, 568–574.
20. Uchikawa, H.; Hanehara, S.; Shirasaka, T.; Sawaki, D. *Cem. Concr. Res.* **1992**, *22*, 1115–1129.
21. Chow, S. *Wood Sci. Technol.* **1983**, *17*, 1–11.
22. Kadla, J.; *Temperature-Modulated Thermogravimetric Analysis of Isolated Lignin*; Abstracts of Papers of the American Chemical Society: CELL Division, March 2003, unpublished data.
23. Sakakira, A. Japan Patent 849,929, 1977.
24. Roffael, E.; Rausch, W. *Holzforschung* **1973**, *27*, 214–217.
25. El Mansouri, N.-E.; Salvadó, J. *Ind. Crop Prod.* **2006**, *24*, 8–16.
26. Gellerstedt, G.; Gustafsson, K. *J. Wood Chem. Technol.* **1987**, *7*, 65–80.
27. Jain, R. K.; Glasser, W. G. *Holzforschung* **1993**, *47*, 325–332.
28. Wu, L. C. F.; Glasser, W. G. *J. Appl. Polym. Sci.* **1984**, *29*, 1111–1123.
29. Wu, L. C. F.; Glasser, W. G. *J. Appl. Polym. Sci.* **1984**, *29*, 1815–1830.
30. Nieh, W. L. S.; Glasser, W. G. In *Lignin: Properties and Materials*; Glasser, W. G., Sarkanen, S., Eds.; ACS Symposium Series 397; American Chemical Society, Washington, DC, 1989; pp 506−514.
31. Jain, R. K.; Glasser, W. G. *Holzforschung* **1985**, *39*, 345–353.
32. Nakamura, K.; Hatakeyama, T.; Hatakeyama, H. *Polym. Adv. Technol.* **1992**, *3*, 151–155.
33. Hirose, S.; Hatakeyama, T.; Hatakeyama, H. *Thermochim. Acta* **2005**, *431*, 76–80.
34. Danielson, B.; Simonson, R. *J. Adhes. Sci. Technol.* **1998**, *12*, 941–946.
35. Danielson, B.; Simonson, R. *J. Adhes. Sci. Technol.* **1998**, *12*, 923–939.
36. Cavdar, A. D.; Kalaycioglu, H.; Hiziroglu, S. *J. Mater. Proc. Technol.* **2008**, *202*, 559–563.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch010

37. Kuo, M.; Hse, C. Y.; Huang, D. H. *Holzforschung* **1991**, *45*, 47–54.
38. Olivares, M.; Guzman, J. A.; Natho, A.; Saavedra, A. *Wood Sci. Technol.* **1988**, *22*, 157–165.
39. Tai, S.; Nakano, J.; Migita, N. *Mokuzai Gakkaishi* **1967**, *13*, 257–262.
40. Feldman, D.; Banu, D.; Natanshon, A.; Wang, J. *J. Appl. Polym. Sci.* **1991**, *42*, 1537–1550.
41. Wang, J.; Banu, D.; Feldman, D. *J. Adhes. Sci. Technol.* **1992**, *6*, 587–598.
42. Nonaka, Y.; Tomita, B.; Hatano, Y. *Holzforschung* **1997**, *51*, 183–187.
43. Simonescu, C. I.; Rusan, V.; Macoveanu, M. M.; Cazacu, G.; Lipsa, R.; Vasile, C.; Stoleriu, A.; Ioanid, A. *Compos. Sci. Technol.* **1993**, *48*, 317–323.
44. Mijovic, J.; Tsay, L. *Polymer* **1981**, *22*, 902–906.
45. Kadla, J.; Kubo, S. *Macromolecules* **2003**, *36*, 7803–7811.
46. Holsopple, D.; Kurple, W.; Kurple, W.; Kurple, K. U.S. Patent 4,265,809, 1981.
47. Glasser, W.; Hsu, O. U.S. Patent 4,017,474, 1977.
48. El Mansouri, N.-E.; Salvadó, J. *Ind. Crop Prod.* **2007**, *26*, 116–124.
49. Vasile, C.; Popescu, M. C.; Stoleriu, A.; Gosselink, R. In *New Trends in Natural and Synthetic Polymer Science*; Vasile, C., Zaikov, G. E., Eds.; Nova Science Publisher: New York, 2006; Ch. 7, pp 135−163.
50. Kubo, S; Kadla, J. F. *Macromolecules* **2004**, *37*, 6904–6911.
51. Thring, R. W.; Vanderlaan, M. N.; Griffin, S. L. *Biomass Bioenergy* **1997**, *13*, 125–132.
52. Hirose, S.; Hatakeyama, T.; Hatakeyama, H. *Macromol. Symp.* **2003**, *197*, 157–169.
53. Rozman, H. D.; Tan, K. W.; Kumar, R. N.; Abubakar, A. *J. Appl. Polym. Sci.* **2001**, *81*, 1333–1340.
54. Kurosumi, A.; Sasaki, C.; Yamashita, Y.; Nakamura, Y. *Trans. Mater. Res. Soc. Jpn.* **2008**, *33*, 1153–1157.
55. Nakamura, Y.; Sawada, T.; Kuno, K.; Nakamoto, Y. *J. Chem. Eng. Jpn.* **2001**, *34*, 1309–1312.
56. Rowell, R. M. *J. Polym. Environ.* **2007**, *15*, 229–235.
57. Karahaliou, E.-K.; Tarantili, P. A. *Polym. Eng. Sci.* **2009**, *49*, 2269–2275.
58. Glasser, W. G. In *Lignin: Historical, Biological, And Materials Perspectives*; Glasser, W. G., Northey, R. A., Schultz, T. P., Eds; ACS Symposium Series 742; American Chemical Society: Washington, DC, 2000; Ch. 9, pp 216−238.
59. Uraki, Y.; Kubo, S.; Nigo, N.; Sano, Y.; Sasaya, T. *Holzforschung* **1995**, *49*, 343–350.
60. Kubo, S.; Ishikawa, N.; Uraki, Y.; Sano, Y. *Mokuzai Gakkaishi* **1997**, *43*, 655–662.
61. Uraki, Y.; Nemoto, J.; Yanaga, K.; Koizumi, A.; Hirai, T. *J. Wood Sci.* **2005**, *51*, 589–594.
62. Glasser, W. G.; Jain, R. K. *Holzforschung* **1993**, *47*, 225–233.
63. Sudo, K.; Shimizu, K.; Nakashima, N.; Yokoyama, A. *J. Appl. Polym. Sci.* **1993**, *48*, 1485–1491.
64. Sudo, K.; Shimizu, K. *J. Appl. Polym. Sci.* **1992**, *44*, 127–134.
65. Kubo, S.; Uraki, Y.; Sano, Y. *Holzforschung* **1996**, *50*, 144–150.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch010

66. Kubo, S.; Uraki, Y.; Sano, Y. *Carbon* **1998**, *36*, 144–150.
67. Kadla, J. F.; Kubo, S; Venditti, R. A.; Gilbert, R. D.; Compere, A. L.; Griffith, W. *Carbon* **2002**, *40*, 2913–2920.
68. Uraki, Y.; Nakatani, A.; Kubo, S.; Sano, Y. *J. Wood Sci.* **2001**, *47*, 465–469.
69. Uraki, Y.; Kubo, S.; Kurakami, H.; Sano, Y. *Holzforschung* **1997**, *51*, 188–192.
70. Kubo, S.; Uraki, Y.; Sano, Y. *J. Wood Sci.* **2003**, *49*, 188–192.
71. Kadla, J. F.; Kubo, S. *Composites, Part A* **2004**, *35A*, 395–400.
72. Uraki, Y.; Usukura, Y.; Kishimoto, T.; Ubukata, M. *Holzforschung* **2006**, *60*, 659–664.
73. Homma, H.; Kubo, S.; Yamada, T.; Matsushita, Y.; Uraki, Y. *J. Wood Chem. Technol.* **2008**, *28*, 270–284.
74. Homma, H.; Kubo, S.; Yamada, T.; Koda, K.; Matsushita, Y.; Uraki, Y. *J. Wood Chem. Technol.* **2010**, *30*, 164–174.
75. Uraki, Y.; Ishikawa, N.; Nishida, M.; Sano, Y. *J. Wood Sci.* **2001**, *47*, 301–307.
76. Bardant, T. B.; Oikawa, C.; Nojiri, M.; Koda, K.; Sudiyanni, Y.; Yamada T.; Uraki, Y. *Mokuzai Gakaishi* 2010, in press.
77. Uraki, Y.; Hashida, K.; Sano, Y. *Holzforschung* **1997**, *51*, 91–97.
78. Uraki, Y.; Hanzaki, A.; Hashida, K.; Sano, Y. *Holzforschung* **2000**, *54*, 535–540.
79. Uraki, Y.; Imura, T.; Kishimoto, T.; Ubukata, M. *Carbohydr. Polym.* **2004**, *58*, 123–130.
80. Uraki, Y.; Imura, T.; Kishimoto, T.; Ubukata, M. *Cellulose* **2006**, *13*, 225–234.

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Chapter 11

Chemicals from Extractives

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Extractives are a minor component of wood but there is a long history of their use as a source of renewable chemicals which have a high value to society. They find uses in products ranging from fragrances, flavors, adhesives, medicines and more recently fuels. The purpose of this chapter is to present an overview of the chemical structure of wood extractives, the procedures used to isolate extractives from wood and their uses. The general structure of the main chemical components of wood extractives, terpenes, rosin, fatty acids and phenolics are given. The common procedures used to isolate the extractive from wood are described along with some of the more common applications. Examples of the chemical modifications used to produce useful derivatives are presented. For each group of extractives, a few new developments at the research stage are discussed.

Introduction

Wood extractives, as the name indicates, are the set of chemicals that can be removed from the woody matrix relatively easily by a solvent extraction process. The solvents acetone, toluene, hexane, ether, ethanol, methanol and water have been commonly used (*1*). Extractives are the secondary metabolites of trees as distinct from the primary metabolites of cellulose, hemicelluloses and lignin. Their presence is one of the ways by which trees protect themselves from attack by pests, bacteria, fungi and viruses (*2*). While the extractives content of wood is low, generally 3 to 5%, they are composed of a diverse set of low molecular compounds whose composition is highly dependent upon the tree species and

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

age. There is a long history of their separation and collection from wood for subsequent use in a wide variety of products. The resin extractives isolated from *Boswellia* and *Commiphora* trees in southern Arabia, incenses frankincense and myrrh respectively, have been used since 1000 BC. Resins for waterproofing wooden ships, "Naval Stores", have been used since 1500 BC. Pine resins were used to waterproof British sailing ships in the 1500s. The production of "Naval Stores" resins and turpentine in the United States peaked in the 1900s. At that time the southern United States became the major supplier of naval stores to the World. Natural rubber has been around since the 1700s and tannins since the 1800s (*3*).

For many years extractives have found applications as dyes, fragrances and medicines. Present day products range from solvents such as turpentine derived from pine, flavors and fragrances obtained from eucalyptus, cedar, pine and rosewood, rosins isolated from softwoods and used in paper sizing, rubber and ink additives. Fatty acids find applications as detergents and are used in ore separation. Phenolics such as flavonoids are used as antioxidants, antifungal agents and insect repellents. Tannins were originally used to tan leather but more recently have found applications as adhesives and medicines. The benefits of extractives in health care and as novel drugs have prompted much recent interest.

In processes designed to obtain chemicals and/or fiber from forest biomass, the early separation of extractives from the fibers, cellulose and lignin could not only provide a stream of valuable low molecular weight chemicals but could also facilitate further processing. Extractives are detrimental in the production of sulfite and mechanical pulps and in the papermaking processes (*4–7*), removal of extractives may increase the efficiency of production of bioethanol (*8*, *9*) and extractives removal is an integral part of char production (*10*).

The diverse chemical structure of wood extractives leads to an enormous range of products and a great potential for the development of new products. In this chapter, an overview of the chemistry, isolation and uses of wood extractives, including some recent trends and developments are presented. For further details the reader is referred to more comprehensive treatments that can be found in publications edited by Belgacem and Gandini (*11*) and Mander and Liu (*12*), Plant Resins authored by Langenheim (*3*) and the classic book on Naval Stores edited by Zinkel and Russell (*13*).

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Chemical Characteristics

Extractives can be classified into four main types of chemicals; terpenes and terpeniods, fats and waxes, phenolics and alkaloids.

Terpenes and Terpenoids

Terpenes are low molecular weight hydrocarbons with structures based on the isoprene (2-methyl-butadiene) unit. They are classified by the number of isoprene units in their structure. Monoterpenes contain two isoprene units (C_{10}), sesquiterpenes 1.5 isoprene units (C_{15}), diterpenes contain four isoprene units

(C_{20}), and triterpenes contain 6 isoprene units (C_{30}). The term terpene generally applies to the pure hydrocarbon. The term terpeniod is used to refer to terpenes that bear one or more functional groups containing oxygen. For example a hydroxyl, a carbonyl or a carboxylic acid group.

Monoterpenes and monotepenoids are volatile compounds and contribute to the characteristic odor of different wood species. Three common monoterpenes, found in pine, are shown in Figure 1 (*14*).

The structures of two common diterpenoids bearing carboxylic acid groups are shown in Figure 2. These acids, found in softwoods, are commonly known as resin acids.

Two triterpenes, bearing secondary alcohol groups, that are found in wood extractives are betulinol and the steroid, sitosterol (Figure 3).

Fats and Waxes

Fats are esters formed from fatty acids and glycerol (triglycerides) and are found in both softwoods and hardwoods ((*1*, *7*, *15*)). More than 30 fatty acids, both saturated and unsaturated, have been identified in wood. Typical fatty acids are palmitic, linoleic, oleic and linolenic acids (Figure 4). Hardwoods also contain significant amounts of waxes. These waxes are esters of fatty acids with fatty alcohols (C_{18-22}) or terpene alcohols (Figure 3).

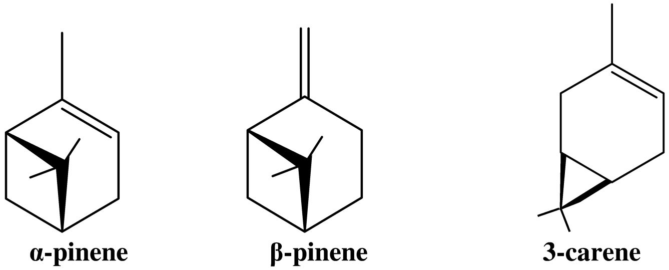

Figure 1. Monoterpenes found in pines

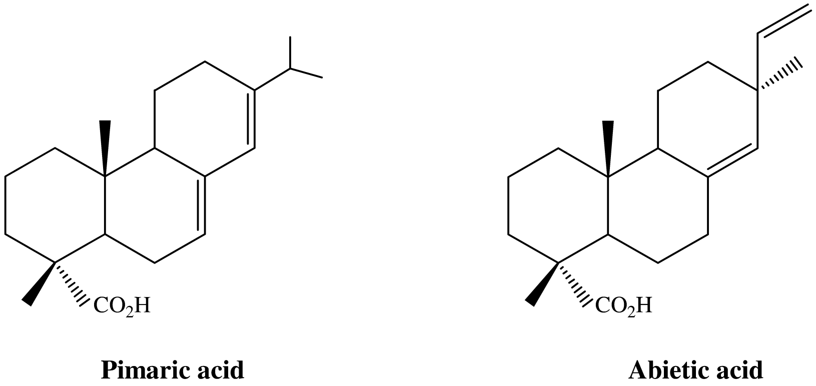

Figure 2. Diterpenoids found in pines and firs

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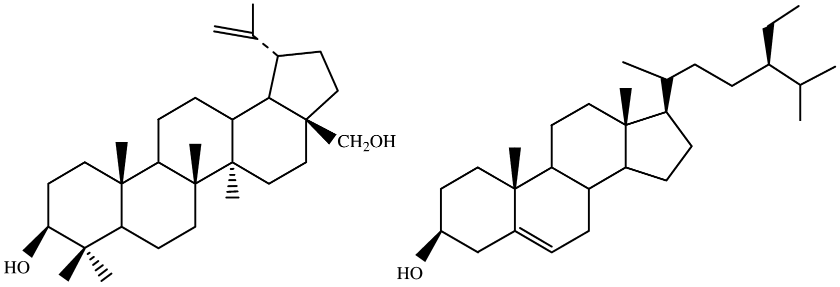
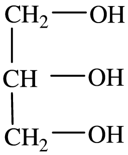


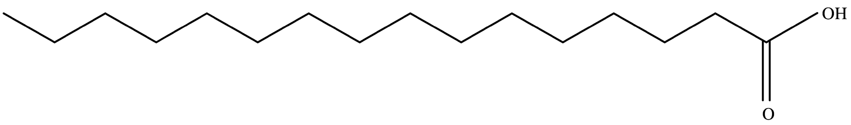
a) Sitosterol **b) Betulinol**

Figure 3. Triterpenoids found in hardwoods (Birch, betula)(a) and softwoods (b)

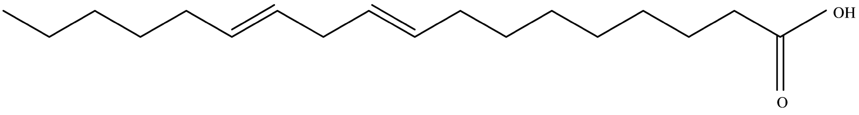


$$\begin{array}{l} CH_2-OH \\ | \\ CH-OH \\ | \\ CH_2-OH \end{array}$$

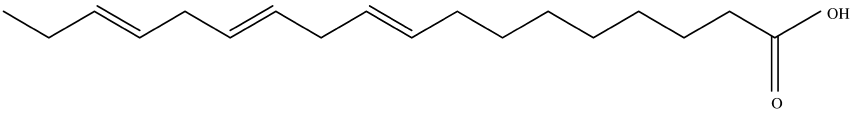
Glycerol



Palmitic acid



Linoleic acid



Linolenic acid

Figure 4. Glycerol and common fatty acids

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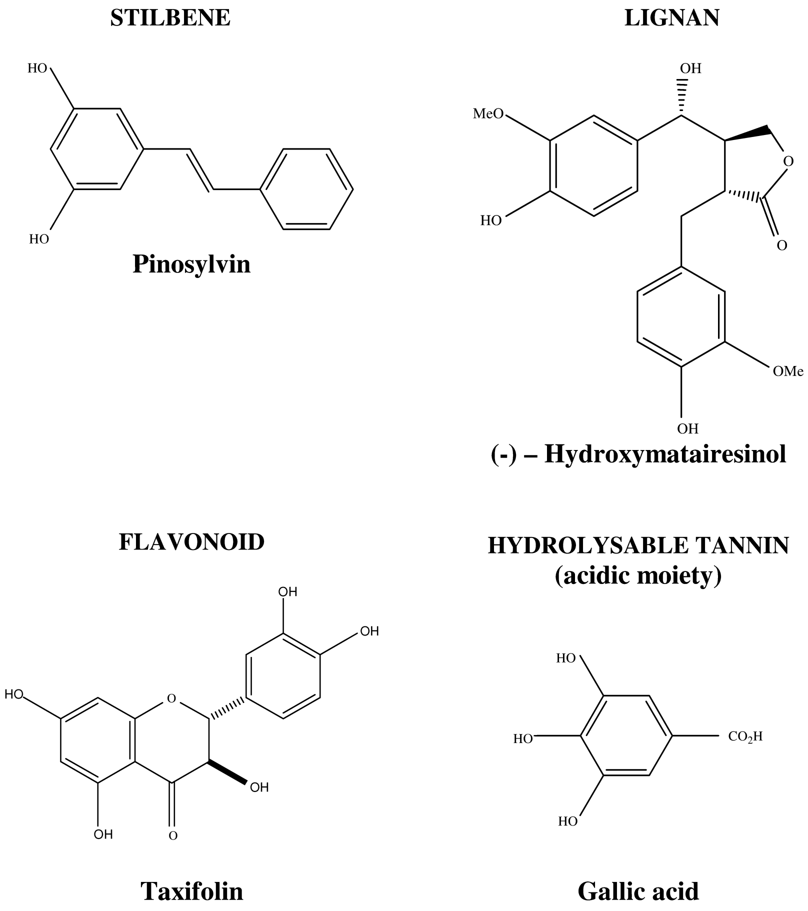
Phenolics

Phenolic compounds are an important and highly variable component of extractives. They are found in five general classes. Simple phenolics, stilbenes, lignans, flavonoids and tannins (hydrolysable and condensed). Phenolic extractives are commonly found in heartwood and bark where their antifungal activity provides protection against microbial attack. Selected examples of the chemical structures of phenolic extractives are shown in Figure 5.



STILBENE

Pinosylvin

LIGNAN

(-) – Hydroxymatairesinol

FLAVONOID

Taxifolin

HYDROLYSABLE TANNIN (acidic moiety)

Gallic acid

Figure 5. Examples of phenolic extractives

Stilbene phenolics are derivatives of 1,2-diphenylethene structures bearing hydroxyl group(s) on the benzene rings and are commonly found in the heartwood of *Pinus* species. Lignans are in the stemwood of both softwoods and hardwoods and are basically formed by oxidative coupling of two phenylpropane units (C_6C_3). Flavonoids are polyphenolics with a $C_6C_3C_6$ skeleton. The flavonoid example given in Figure 5, Taxifolin (dihydroquercetin), is found in the heartwood of Douglas fir. Polymers of flavonoids are called condensed tannins. Hydrolyzable tannins are esters of sugars such as D-glucose and polyphenolic acids, gallic or ellagic acid, which are readily hydrolyzed by acid, alkali and enzymes such as tannase.

Alkaloids

Alkaloids, are a highly variable and structurally complex yet important class of extractives mainly found in the bark and leaves of trees. They are toxic and believed to function as defenses against predators especially mammals. They contain nitrogen and are usually derived from amino acids such as lysine, tyrosine or tryptophan. Others are derived from ornithine. Some, for example Taxol, contain components derived via the terpene pathway (*16*).

Isolation and Purification of Extractives from Woody Biomass

Large scale isolation of extractives from wood is accomplished in several ways. Traditional procedures involve tapping trees to obtain resins and latex. Distillation of resin yields a volatile fraction, turpentine and a residue, rosin. Other processes utilize steam distillation or solvent extraction of wood and bark. Since the 1950's turpentine, rosin and fatty acids have been isolated as a by-product of the kraft pulping process particularly of pine. The rosin and turpentine obtained by the different processes of tree tapping, steam distillation of wood and kraft pulping are distinguished from one another by different nomenclature.

Steam distillation or solvent extraction of the heartwood of pine provides a volatile fraction, mainly composed of terpenes, which is called wood turpentine, plus a residue named wood rosin, mainly consisting of resin acids. The tapping process yields the so called gum resin which is then steam distilled to yield gum turpentine and gum rosin (*17*). In the kraft process, as wood chips are pre-heated with steam prior to cooking, the volatiles emitted are cooled to form turpentine (sulfate turpentine). The organic sulfur impurities, largely methyl mercaptan and dimethylsulfide and higher terpenes are removed in a distillation step (*7*). After the wood chips have been cooked in the alkaline liquor at 170°C for about 90 minutes the lignin, hemicelluloses and extractives are dissolved in the "black liquor". The black liquor exits the digester at around 16% solids. This liquor is evaporated until the solids content is around 25% at which time crude tall oil is skimmed off the surface of the black liquor as a soap ("soap skimmings"). The soap is acidified with sulfuric acid to a pH of 4 converting the sodium salts to their carboxylic acid form. At this time sodium salts of lignin, entrained in the soap, precipitate out forming an emulsion between the oil and aqueous layers. Separation of the layers

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yields the crude tall oil. Yields of 2-4% of crude tall oil based on air dried pulp production are common (*18*, *19*). Distillation of the crude tall oil gives four main fractions, a light oil (10-15%), fatty acids (20-40%), rosin (25-35%) and a pitch (20-30%). The rosin and fatty acids produced in this manner are the so called sulfate rosin and sulfate fatty acids. Removal of the neutrals from the resin and fatty acids by extraction, improves their quality and provides a sterol rich stream that can be further utilized (*7*, *20*).

Uses of Wood Extractives

The utilization of extractives from the forest biomass has a long history and continues to evolve. In this section, the traditional uses of extractives are reviewed and some of the newer developments are summarized.

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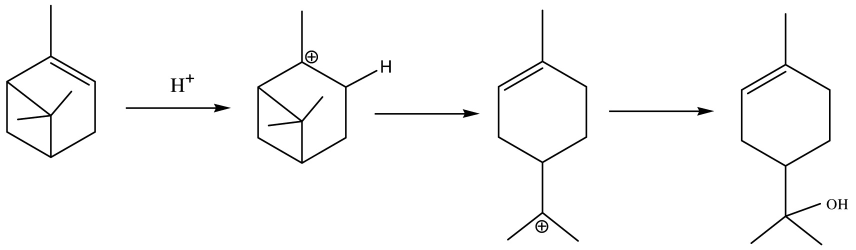
Turpentine

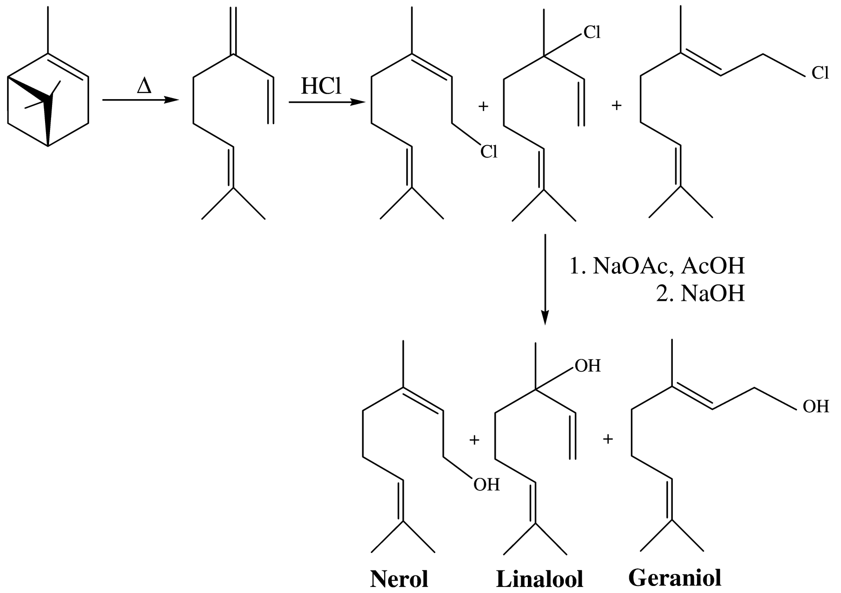
Turpentine can be obtained by distillation of the resin extracted by tapping pines and other conifers such as spruce and balsam. The composition of the turpentine is variable and dependent upon species. Turpentine obtain by this method is known as gum turpentine. Turpentine can also be obtained by direct solvent extraction of harvested wood: wood turpentine. Presently most of the turpentine, sulfate turpentine, is a by-product of the kraft pulping process. Both pine and spruce give good turpentine yields. The sulfate turpentine from the Southeast USA consists mainly of α-pinene (60 – 75%) and β-pinene (20-25 %). Turpentine from Sweden and Russia contains significant amounts of 3-carene (≈ 40%) (*21*). Small amounts of other terpenes such as limonene and camphene, and terpene oxidation products such as aldehydes and alcohols, can be present. Turpentine production has been estimated at 335,000 tonnes per year, about 100,000 tonnes per year from gum turpentine most of the rest being sulfate turpentine (*17*, *22*).

Turpentine was long used as a solvent, above all for paints, but its use for this purpose has diminished in favour of non-renewable petroleum based solvents. The latter are often called mineral turpentine to distinguish them from the turpentines isolated from wood. The major use of turpentine is now as a raw material for chemical manufacture. Products manufactured from turpentine include pine oil, flavours, fragrances, pharmaceuticals and polyterpene resins (*23*). Approximately 47% of the turpentine produced is used to make solvents, 36% goes into flavours and fragrances and the remaining 17% is used in the manufacture of polyterpene resins (*3*). Derivatives such as isobornyl acetate, camphor, citral, linalool, citrinellal, menthol and many others are used either in their own right or for the elaboration of other fragrance and flavor compounds. Many of the odors and flavors in use today, which are associated with naturally occurring oils, may well be derived, instead, from turpentine. However, many of the odorants and flavouring agents traditionally produced from terpenes extracted from turpentine have been replaced by cheaper petroleum based versions. The chemistry behind the conversion of wood derived terpenes into useful products is exemplified in the following examples.

Alpha-pinene is a major constituent of turpentine and is the starting point for a diverse array of products. The main product continues to be synthetic pine oil, used for cleaning and disinfection. Synthetic pine oil is prepared through hydration of α-pinene using aqueous mineral acids. The first step is the formation of a carbocation, which after isomerisation and addition of water forms α-terpineol (Scheme 1).



Scheme 1. Conversion of α-pinene into synthetic pine oil



Scheme 2. Synthesis of nerol, linalool and geraniol from β-pinene

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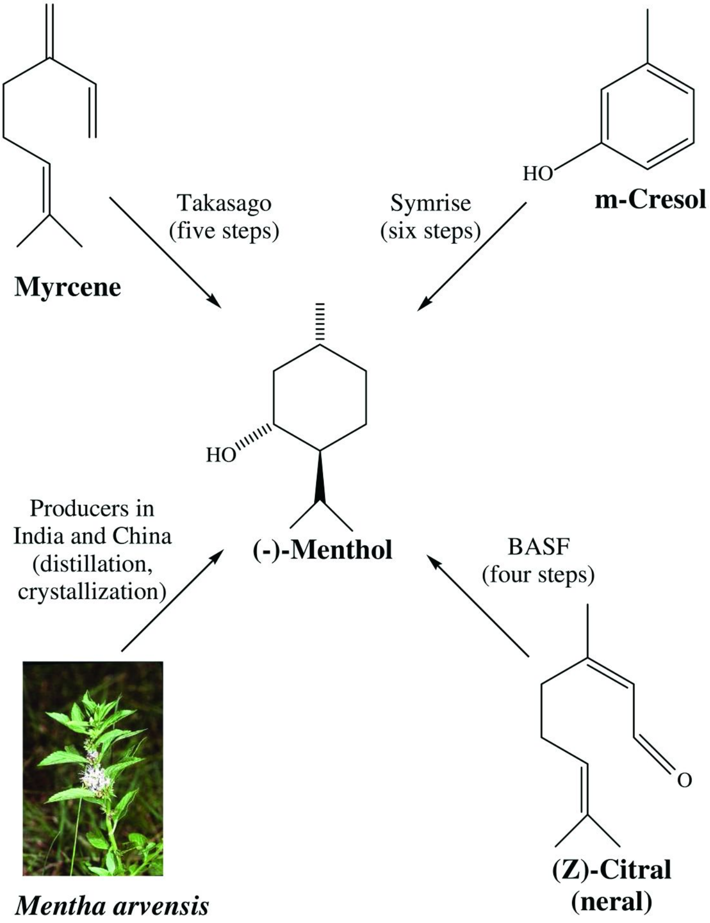
Myrcene
Takasago
(five steps)
Symrise
(six steps)
HO
m-Cresol
HO
(-)-Menthol
Producers in
India and China
(distillation,
crystallization)
BASF
(four steps)
O
Mentha arvensis
(Z)-Citral
(neral)

Scheme 3. Menthol made via four competing routes. Reprinted from with permission from (25). Copyright (2010) ACS. Image by Robert Tatina @ USDA-NRCS Plants database (28) (see color insert)

Representative of the use of turpentine as a feedstock for manufacture of fragrances is the conversion of β-pinene to the rose alcohols, nerol, geraniol and linalool which are key intermediates in many major fragrances. This is accomplished through isomerisation of β-pinene to myrcene, followed by addition of hydrogen chloride, substitution of the chlorine with acetate followed by alkaline hydrolysis to yield the alcohols (Scheme 2). The alcohol linalool is important as it is an intermediate in the manufacture of Vitamin E. Linalool can also be produced from α-pinene through hydrogenation to pinane, oxidation of pinane to pinane hydroperoxide, reduction of pinane to pinanol followed by pyrolysis to linalool (*3*, *24*). Another important chemical prepared industrially from α-pinene is camphor, which is used as a flavorant, moth-repellant, plasticizer and preservative (*14*).

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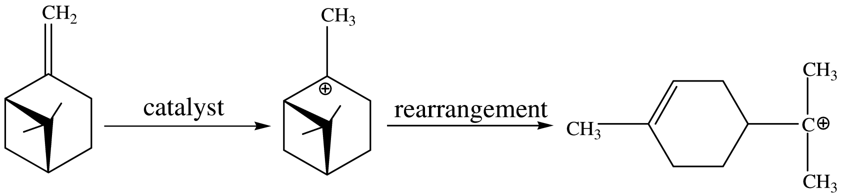
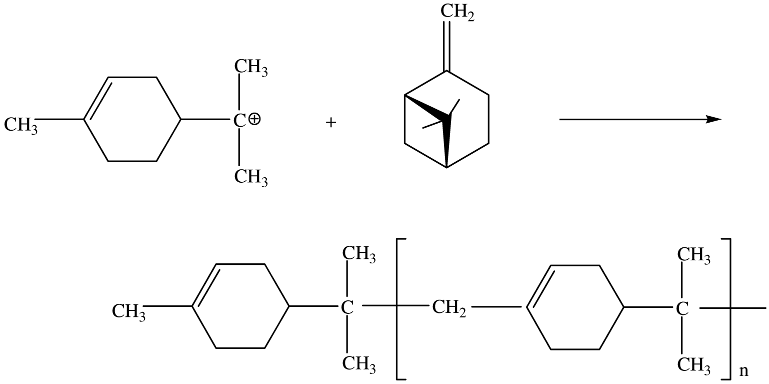


Initiation of β-pinene polymerization



Propagation of β-pinene polymerization

Scheme 4. Polymerization of β-pinene

Myrcene is starting point for the production of menthol. Menthol is an important chemical used in toothpaste, cigarettes, cough drops and other consumer products. The Global demand is over 20,000 metric tons per year selling for around \$19 per kg (*25*). It provides an excellent example of a product that is derived directly from plants, from terpenes and petroleum based chemicals. Menthol can be obtained from *Mentha arvensis* by distillation and crystallization, synthetically from *m*-cresol, obtained from coal tar or petroleum, from the terpene myrcene and from citral synthesized from isobutylene and formaldehyde (*26*, *27*) (Scheme 3).

It is apparent that terpenes from woody biomass provide a rich source of many chemicals which have the potential of replacing those presently derived from fossil resources. Furthermore, with renewed research in terpene applications this could be expanded beyond the traditional applications. Recently, research has been conducted into the use of turpentine as an additive for gasoline (*29*).

Terpenes undergo cationic polymerization to provide low molecular weight polymers (Scheme 4). These polymers find applications as tackifiers in adhesives and additives to improve polymer properties such as flexibility and the vapor transmission rates of wax coatings (*23*, *30*).

Polyterpenes synthesized from sulfate turpentine have been used to prepare composites with adhesive and varnish properties for protection of electric circuits and also copolymers for printing ink formulations and protective coatings (*31*). The chemistry of preparation of polymers from terpenes has been extensively reviewed (*23*). Potentially biodegradable polymers based on pinene and sugar derivatives have recently been prepared (*32*).

Terpenoids (Rosin)

Rosin has traditionally been obtained as the residue remaining after the removal of the volatile terpenes from the exudates of pine trees (gum rosin) or from the solvent extract of pine trees (wood rosin). Presently, the major source of rosin is the tall oil rosin separated from crude tall oil collected during the kraft pulping process (see above).

The principle components of the rosin are resin acids of the pimaric and abietic type (Figure 2). The presence of both hydrophilic and hydrophobic sections in these molecules gives rosin the properties required in major applications related to waterproofing and water repellency. A major end use is paper sizing, the control of water penetration into paper. In this process the rosin is attached to the negatively charged fiber surface through a bridging with alum to the carboxylic acid group on the rosin (Figure 6).

The efficiency of the rosin as a paper sizing agent can be increased by attaching further carboxylic acid groups through a Diels alder reaction with maleic anhydride.

The use of rosin in paper sizing has decreased in recent years as it requires a pH in the range of 4-5.5 during the papermaking process. Such a low pH is incompatible with the commonly used cheap calcium carbonate based fillers. Replacement sizes such as alkyl ketene dimers and alkenyl succinic anhydrides covalently bond to the fiber surface and are effective at neutral pH. However, current research efforts are underway to develop alum-free systems that make rosin size compatible with calcium carbonate fillers. Such systems use polyamines and polyaluminum systems for retention of the rosin to the fibers under neutral – alkaline conditions (*33*, *34*).

Other major uses of rosin and rosin derivatives are as tackifiers in adhesives, emulsifiers in synthetic rubber, in printing inks and coatings, and in chewing gum (*3*, *35*, *36*).

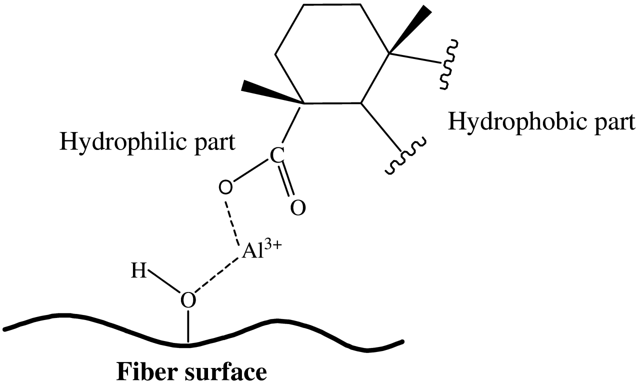


Figure 6. Mechanism of paper sizing

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Terpenoids (Phytosterols and Phytostanols)

The forest is a source of sterols that are increasingly finding application in cholesterol reducing foods. These sterols, such as β-sitosterol (Figure 3), can be isolated on a large scale from the neutral fraction of tall oil (see above) (*3*). Catalytic hydrogenation of β-sitosterol provides β-sitostanol (*37*) which as an ester, can be readily incorporated into fatty foods. The FDA has approved use of up to 20% of these esters in spreads. Benecol, which contains 8% of a stanol ester has been on the market since 1999. Tall-oil derived phytosterols have also been shown to be effective in reducing cholesterol levels when used as an additive in chocolate (*38*).

Much recent interest has been the application of triterpenoids in medical fields. Triterpenoids such as betulinic acid, which can be obtained from the bark of the London plane tree, and Boswellic acids, isolated from *Boswellia serrata*, show significant activity against various cancers and against HIV (*39*, *40*).

Fatty Acids

Fats and waxes from plants are mainly used for human consumption but approximately 15% or around 15.6 million tons are used as precursors to useful renewable chemicals. They have traditionally found industrial applications in paints and coatings, lubricants, agrochemicals and plasticizers (*41*). The plant derived fats and waxes, triglycerides and terpenoid esters of fatty acids, are mainly obtained from the seeds of plants such as soya bean oil, palm oil and rapeseed/canola oil and linseed (*41*). However, as discussed above, fatty acids are also obtained from the forest biomass as a fraction during distillation of crude tall oil, of which they constitute around 30%. Commonly they consist mainly of oleic, linolenic and palmitic acids (Figure 4) (*15*, *41*). Fatty acids find major uses in the manufacture of surface-active agents and in coating. They are also used in oil well drilling and for formulation of lubricants, greases and anti-corrosives. In the pharmaceutical industry they find use in the production of water-oil emulsions. The alkali salts are used as soaps but have to a great extent been replaced by synthetic detergents. Esters of the tall oil fatty acids with polyols are used in alkyd resins and as drying oils and surfactants. Esters with monohydric alcohols find use as plasticizers and stabilizers for plastics. These traditional applications are well covered by Mattson (*42*) and Logan and Ennor (*43*) and more recent advances, especially in the production of polymeric materials from trigycerides, are covered by Belgacem (*41*).

Fatty acids esters provide the basis for preparation of biodiesel. Rudolph Diesel realized more than a century ago that vegetable oils could be used to run engines (*44*). Biodiesel is generally formed by transesterification of the triglycerides with methanol under alkaline conditions in the presence of catalysts such as zeolites, calcium compounds and enzymes (Scheme 5)(*45*).

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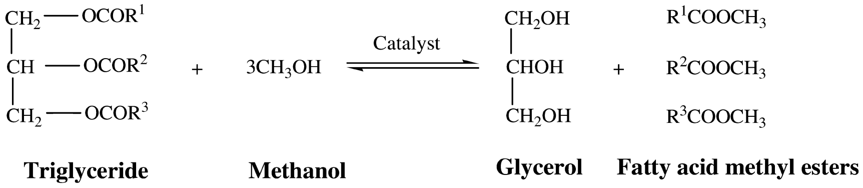

Scheme 5. Transesterification of triglycerides

Biodiesel generated from relatively pure vegetable oil trigycerides can be added to petro diesel and used without engine modification generally resulting in lower emissions. One issue is the low temperature performance of biodiesel. The esters of fatty acids tend to crystallize at low temperatures. This can limit their use in blends with petrodiesel to less than 20%. A further issue is the tendency of the unsaturated ester to polymerize and form gums.

Tall oil provides an alternative source of the free fatty acids which can be esterified with methanol to give biodiesel. The potential of various methods of generating biodiesel from tall oil have recently be assessed by Lee at al (*45*). The authors considered the pros and cons of using acid, alkaline and enzymatic catalysis and the use of supercritical methanol. Recent trials have assessed the performance of mixtures of biodiesel from tall oil and petrodiesel in engines (*46*). In some trials metallic fuel additives generated from tall oil resin were also utilized (*47*, *48*). Advantages such as lower CO and NOx emissions were observed, although specific fuel consumption was higher by about 5%. It was also noted that the crude tall oil used as the starting material for the work was 60% cheaper than vegetable oils.

Phenolics

The diversity of phenolic extractives, found mainly in the bark and heartwood of trees, presents a source of a wide range of compounds with many applications. The phenolic nature of these extractives makes them attractive as feedstocks for biobased phenolic polymers. Their ability to protect the tree from attack by insects and micro-organisms makes them candidates for anti-fungal agents and insecticides. Other applications in the medicinal field arise from their antioxidant activity. The following examples serve to demonstrate the use of phenolic wood extractives in each of these areas.

Tannins are found in high concentrations in the bark of conifers and eucalypts and have long being used in the leather tanning process. However, in this application they have lost market share to synthetic tannins and now make up only 10-20% of the total tanning market. Of the commercially produced tannins, over 90% are condensed tannins (*49*, *50*). The availability of waste bark from wood processing and the desire for products manufactured from renewable resources has led to research and development into other uses for tannins. One such use, already established, is as an adhesive in particleboards and medium density fiberboard (*51*, *52*). In this application, at higher pH, the free position (6) on ring ‘A’ between the vicinal hydroxyl groups becomes highly nucleophilic on generation of the phenateion and it reacts rapidly with formaldehyde (Figure 7) (*49*).

Research activity continues into developing adhesives based on condensed tannins extracted from different species, such as *Acacia mearnsii* (Black Wattle), *Pinus pinaster* (maritime pine) and *Pinus radiata* (radiata pine) (*53–57*). An interesting recent development is the preparation of renewable composite material consisting of a tannin-phenolic polymer reinforced with coir fibers obtained from coconut (*58*).

Other uses for tannins are found in the areas of polyurethane production (*59*) and production of rigid foams (*60*, *61*). These foams, formed from furfuryl alcohol, formaldehyde and tannin extract, display properties similar to industrial phenol-formaldehyde (phenolic resoles) foams.

Plant polyphenols such as flavonoids, lignans and condensed tannins possess both antioxidant, biocidal and chelating activities which has led to extensive investigation into in their efficacy in the field of medicine, to provide protection against cancer and heart disease, and also to their use for fungal and bacterial control (*62–68*). Holmbom et al have both chemically characterized and assessed the anti-oxidant potency of extracts from bark and knots from a wide selection of species (*69–71*). Both knots and bark are available in large quantities as waste in the wood processing and pulp and paper industries. Holmbom found that knotwood extracts from *Abies, Larix, Picea* and *Tsuga* were rich in lignans and oligolignans (lignans with 3 to 6 β-β linked phenylpropane units) whereas in *Pinus* species pinosylvins dominated. Flavonoids dominated in *Populus* and *Acacia* knotwood extracts. The bark extracts were rich in tannins. The knotwood extracts were found to be stronger antioxidants than both the bark extracts and the respective individual chemical components. Spruce knots provide a source of hydroxymatairesinol (HMR) (Figure 5) which has strong anti-oxidant properties and can inhibit cancer growth. It should be noted that HMR was approved as a dietary supplement by the UDSA in 2004 and the industrial production of HMR from Norway spruce knots began in 2006.

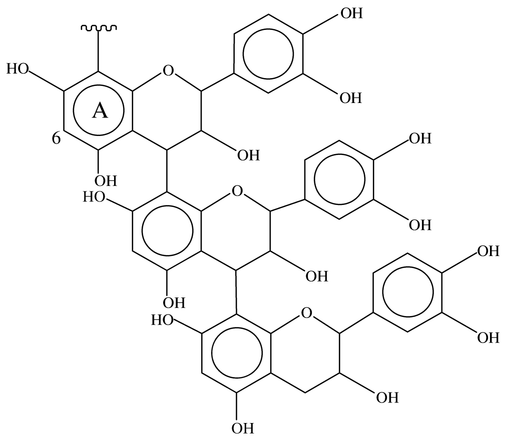

Figure 7. Condensed tannin from pine

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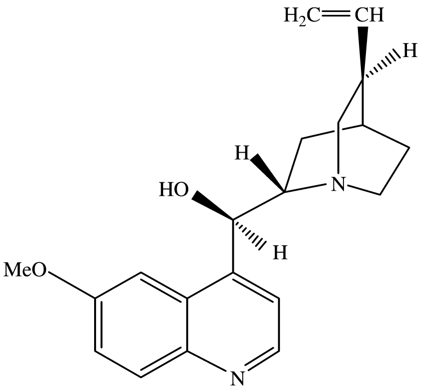

Figure 8. Quinine

Alkaloids

Alkaloids isolated from trees have provided a source of medicinals for a long time. This topic is too large to cover in any detail in a short review and the reader is referred to the recent chapter by Patten et al. in Comprehensive Natural Products II (*72*) and references quoted therein. Perhaps one of the most famous alkaloid is quinine (Figure 8) which was first isolated from the bark of the *Chincona* tree in 1820 and subsequently was extensively used to combat malaria. As with the case of other successful wood derived chemicals, its very success led to a search for and subsequent production of synthetic alternatives. In this case synthetic antimalarial drugs based on the quinolene nucleus were developed (*73*).

Taxol also demonstrates the transition from an initial success of a wood extractive leading to an intensive search for synthetic alternatives. Taxol isolated from the bark of the Pacific yew (*Taxus brevifolia*) was discovered by Wall and Wani in 1971 (*74*). Taxol suppresses mitosis during cell division finding use in treatment of aggressive cancers of the breast, lung, and ovaries (*72*). Its success led to a demand for a production rate of around 1 ton per year which could not be viably sustained by harvesting the Pacific yew. This resulted in the present production from baccatin III isolated from the needles of the European yew tree. Efforts are now underway to find a biosynthetic route to Taxol using engineered bacteria (*75*, *76*).

Concluding Remarks

Wood extractives provide a source of a wide range of chemicals with many applications such as surfactants, polymers, coatings, water repellants, flavor additives, perfumes and medicants. Many of the applications and uses have been in existence for a long time and have suffered from strong competition from non-renewable alternatives based on fossil resources. However, the increasing emphasis on the use of renewable resources as a source of chemicals and fuels has sparked renewed interest in the development of products from wood extractives. Separation of extractives as a step in processes leading to other products such as lumber, pulp, biofuels and lignocellulosic based chemicals would not only provide increased value from the biorefinery but also could alleviate downstream problems associated with the presence of extractives. Increasing utilization of renewable resources as a chemical feedstock could encourage further separation of tall oil from kraft black liquor rather than continued incineration in the recovery furnace. Rather than just burning wood wastes such as bark and knots, why not first extract and utilize these valuable chemical components? As more fuels, polymers and chemicals are produced from the main wood components, cellulose, lignin and hemicelluloses, increased opportunities arise to integrate harvesting and utilization of the extractives into these new processes.

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References

1. Alén, R. In *Forest Products Chemistry*; Stenius, P., Ed.; Papermaking Science and Technology; Fapet Oy: Helsinki, Finland, 2000; Vol. 3, pp 12−57.
2. Turley, D. B.; Chaudhry, Q.; Watkins, R. W.; Clark, J. H.; Deswarte, F. E. I. *Ind. Crops Prod.* **2006**, *24* (3), 238–243.
3. Langenheim, J. H. *Plant Resins: Chemistry, Evolution, Ecology, and Ethnobotany*; Timber Press: Portland, OR, 2003.
4. Johansson, C. I.; Beatson, R. P.; Saddler, J. N. *Wood Sci. Technol.* **2000**, *34* (5), 389–401.
5. Zhang, X.; Nguyen, D.; Paice, M. G.; Tsang, A.; Renaud, S. *Enzyme Microb. Technol.* **2007**, *40* (4), 866–873.
6. Zhang, X.; Stebbing, D. W.; Saddler, J. N.; Beatson, R. P.; Kruus, K. *J Wood Chem. Technol.* **2000**, *20* (3), 321–335.
7. SjöströmE. *Wood Chemistry: Fundamentals and Applications*; Academic Press: San Diego, CA, 1993.
8. Klinke, H. B.; Thomsen, A. B.; Ahring, B. K. *Appl. Microbiol. Biotechnol.* **2004**, *66* (1), 10–26.
9. Tran, A. V.; Chambers, R. P. *Biotechnol. Lett.* **1985**, *7* (11), 841–845.
10. Demirbas, A. *Energy Convers. Manage.* **2009**, *50* (11), 2782–2801.
11. Belgacem, M. N.; Gandini, A. *Monomers, Polymers and Composites from Renewable Resources*; Elsevier: Amsterdam, Holland, 2008.
12. Mander, L.; Lui, H., Eds.; *Comprehensive Natural Products II. Chemistry and Biology*; Elsevier Science and Technology: 2010.
13. Zinkel, D. F.; Russell, J., Eds.; *Naval Stores: Production, Chemistry, Utilization*; Pulp Chemicals Association: New York, 1989.
14. Breitmaier, E. *Terpenes: Flavors, Fragrances, Pharmaca, Pheromones*; Wiley-VCH: Weinheim, Germany, 2006.
15. Duncan, D. P. In *Naval Stores: Production, Chemistry, Utilization*; Zinkel, D. F., Russell, J., Eds.; Pulp Chemicals Association: New York, 1989; pp 346−439.
16. Taiz, L.; Zeiger, E. *Plant Physiology*; 4th ed.; Sinauer Associates: Sunderland, MA, 2006; pp 315−344.

17. Coppen, J. J. W.; Hone, G. A. *Gum Naval Stores: Turpentine and Rosin from Pine Resin*; Non-Wood Forest Products 2; FAO; Rome, Italy, 1995.
18. McSweeney, E. E. In *Naval Stores: Production, Chemistry, Utilization*; Zinkel, D. F., Russell, J., Eds.; Pulp Chemicals Association: New York, 1989; pp 158−199.
19. Tall Oil Production and Processing. http://nzic.org.nz/ChemProcesses/forestry/4G.pdf.
20. Fernandes, P.; Cabral, J. M. S. *Bioresour. Technol.* **2007**, *98* (12), 2335–2350.
21. Turpentine, 1994. http://apps.kemi.se/flodessok/floden/kemamne_eng/terpentin_eng.htm.
22. Ciesla, W. M. *Non-Wood Forest Products from Conifers*; Non-Wood Forest Products 12; FAO: Rome, Italy, 1998.
23. Silvestre, A. J. D.; Gandini, A. In *Monomers, Polymers and Composites from Renewable Resources*; Belgacem, M. N.; Gandini, A., Eds.; Elsevier: Amsterdam, Holland, 2008; pp 17−38.
24. Surburg, H.; Panten, J. *Common Fragrance and Flavor Materials*, 5th ed.; Wiley-VCH: Weinheim, Germany, 2006.
25. McCoy, M. *Chem. Eng. News* **2010**, *88* (35), 15–16.
26. Bergner, E. J.; Ebel, K.; Johann, T.; Löber, O. U.S. Patent 7709688 B2, 2010.
27. Menthol Information. http://www.leffingwell.com/menthol1/menthinfo.htm.
28. The Plants Database, 2011. http://plants.usda.gov.
29. Yumrutas, R.; Alma, M. H. I.; Özcan, H.; Kaska, Ö. *Fuel* **2008**, *87* (2), 252–259.
30. Ruckel, E. R.; Arlt, H. G., Jr. In *Naval Stores: Production, Chemistry, Utilization*; Zinkel, D. F.; Russell, J., Eds.; Pulp Chemicals Association: New York, 1989; pp 510−530.
31. Cascaval, C. N.; Ciobanu, C.; Rosu, L.; Rosu, D. *Mater. Plast.* **2005**, *42* (3), 229–232.
32. Barros, M. T.; Petrova, K. T.; Ramos, A. M. *Eur. J. Org. Chem.* **2007**, *2007* (8), 1357–1363.
33. Zou, Y.; Hsieh, J. S.; Wang, T. S.; Mehnert, E.; Kokoszka *Tappi J.* **2004**, *3*, E41–59.
34. Wang, F.; Tanaka, H. *J. Appl. Polym. Sci.* **2000**, *78* (10), 1805–1810.
35. Silvestre, A. J. D.; Gandini, A. In *Monomers, Polymers and Composites from Renewable Resources*; Belgacem, M. N.; Gandini, A., Eds.; Elsevier: Amsterdam, Holland, 2008. pp 67−88.
36. Chen, G. *Prog. Org. Coat.* **1992**, *20* (2), 139–167.
37. Murzin, D. Y.; Mäki-Arvela, P; Salmi, T.; Holmbom, B. *Chem. Eng. Technol.* **2007**, *30* (5), 569–576.
38. de Graaf, J.; de Sauvage Nolting, P. R. W.; van Dam, M.; Belsey, E. M.; Kastelein, J. J. P.; Pritchard, P. H.; Stalenhoef, A. F. H. *Br. J. Nutr.* **2002**, *88* (05), 479–488.
39. Kuo, R. Y.; Qian, K.; Morris-Natschke, S. L.; Lee, K. H. *Nat. Prod. Rep.* **2009**, *26* (10), 1321–1344.
40. Shah, B. A.; Qazi, G. N.; Taneja, S. C. *Nat. Prod. Rep.* **2009**, *26* (1), 72–89.

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41. Belgacem, M. N.; Gandini, A. In *Monomers, polymers and Composites from Renewable Resources*; Belgacem, M. N., Gandini, A., Eds.; Elsevier: Amsterdam, Holland, 2008; pp 39−65.
42. Mattson, R. H. In *Naval Stores: Production, Chemistry, Utilization*; Zinkel, D. F., Russell, J., Eds.; Pulp Chemicals Association: New York, 1989; pp 741−779.
43. Logan, R. L.; Ennor, K. S. In *Naval Stores: Production, Chemistry, Utilization*; Zinkel, D. F., Russell, J., Eds.; Pulp Chemicals Association: New York, 1989, pp 780−799.
44. Meher, L. C.; Vidya Sagar, D.; Naik, S. N. *Renewable Sustainable Energy Rev.* **2006**, *10* (3), 248–268.
45. Lee, S. Y.; Hubbe, M. A.; Saka, S. *BioResources* **2006**, *1* (1), 150–171.
46. Altiparmak, D.; Keskin, A.; Koca, A.; Gürü, M. *Bioresour. Technol.* **2007**, *98* (2), 241–246.
47. Keskin, A.; Gürü, M.; Altiparmak, D. *Fuel* **2007**, *86* (7-8), 1139–1143.
48. Keskin, A.; Gürü, M.; Altiparmak, D. *Energy Convers. Manage.* **2010**, *52* (1), 60–65.
49. Pizzi, A. In *Wood Adhesives: Chemistry and Technology*; Pizzi, A., Ed.; Marcel Dekker Inc.: New York, 1983; pp 178−248.
50. A Historic Perspective on Tannin Use, 2009. http://www.natural-specialities.com/natural-specialities/.
51. Roffael, E.; Dix, B.; Okum, J. *Eur. J. Wood Wood Prod.* **2000**, *58* (5), 301–305.
52. Pizzi, A. In *Wood Adhesives: Chemistry and Technology*; Pizzi, A., Ed.; Marcel Dekker, Inc.: New York, 1989; pp 177−246.
53. Kim, S.; Kim, H. J. *J. Adhes. Sci. Technol.* **2003**, *17* (10), 1369–1383.
54. Kim, S.; Lee, Y. K.; Kim, H. J.; Lee, H. H. *J. Adhes. Sci. Technol.* **2003**, *17* (14), 1863–1875.
55. Lopez-Suevos, F.; Riedl, B. *J. Adhes. Sci. Technol.* **2003**, *17* (11), 1507–1522.
56. Ohara, S. *Japan Agric. Res. Q.* **1994**, *28*, 70–78.
57. Vázquez, G.; González-Álvarez, J.; Antorrena, G. *J. Therm. Anal. Calorim.* **2006**, *84* (3), 651–654.
58. Barbosa, V., Jr.; Ramires, E. C.; Razera, I. A. T.; Frollini, E. *Ind. Crops Prod.* **2010**, *32*, 305–312.
59. Hartman, S. U.S. Patent 4,032,483, 1977.
60. Tondi, G.; Pizzi, A. *Ind. Crops Prod.* **2009**, *29* (2−3), 356–363.
61. Tondi, G.; Zhao, W.; Pizzi, A.; Du, G.; Fierro, V.; Celzard, A. *Bioresour. Technol.* **2009**, *100* (21), 5162–5169.
62. Swanson, C. A. In *Phytochemicals: A New Paradigm*; Bidlack, W. R., Omaye, S. T., Meskin, M. S., Jahner, D., Eds.; Technomic: Lancaster, PA, 1998; pp 1−12.
63. German, J. B.; Dillard, C. J. In *Phytochemicals: A New Paradigm*; Bidlack, W. R., Omaye, S. T., Meskin, M. S., Jahner, D., Eds.; Technomic: Lancaster, PA, 1998; pp 13−32.
64. Gross, G. G., Hemingway, R. W., Yoshida, T., Branham, S. J., Eds.; *Plant Polyphenols 2*; Kluwer/Plenum: New York, 1999.

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65. Heim, K. E.; Tagliaferro, A. R.; Bobilya, D. J. *J. Nutr. Biochem.* **2002**, *13* (10), 572–584.
66. Stevenson, D. E.; Hurst, R. D. *Cell. Mol. Life Sci.* **2007**, *64* (22), 2900–2916.
67. Verma, A. K.; Pratap, R. *Nat. Prod. Rep.* **2010**, *27* (11), 1571–1593.
68. Phelan, M.; Aherne, S. A.; Wong, A.; O'Brien, N. M. *J. Med. Food* **2009**, *12* (6), 1245–1251.
69. Lignans and Other Bioactive Polyphenols in Wood and Bark of Nordic Trees, 2010. http://biorefinery2010.funcfiber.se/.
70. Holmbom, B.; Willför, S.; Hemming, J.; Pietarinen, S.; Nisula, L.; Eklund, P.; Sjöholm, R. In *Materials, Chemicals and Energy from Forest Biomass*; Argyropoulos, D. S., Ed.; ACS Symposium Series 954; American Chemical Society: Washington, DC, 2007, pp 350−362.
71. Pietarinen, S. P.; Willför, S. M.; Ahotupa, M. O.; Hemming, J. E.; Holmbom, B. R. *J. Wood Sci.* **2006**, *52* (5), 436–444.
72. Patten, A. M.; Vassao, D. G.; Wolcott, M. P.; Davin, L. B.; Lewis, N. G. In *Comprehensive Natural Products II*; Mander, L., Lui, H., Eds.; Elsevier Science and Technology: New York, 2010; pp 1173−1296.
73. Greenwood, D. *Antimicrobial Drugs: Chronicle of a Twentieth Century Medical Triumph*; Oxford University Press: Oxford, UK, 2008.
74. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93* (9), 2325–2327.
75. Ajikumar, P. K.; Xiao, W. H.; Tyo, K. E. J.; Wang, Y.; Simeon, F.; Leonard, E.; Mucha, O.; Phon, T. H.; Pfeifer, B. *Science* **2010**, *330* (6000), 70–74.
76. *Chem. Eng. News* **2010**, *88* (40), 6.

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Chapter 12

Development and Properties of Nanocrystalline Cellulose

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Nanocrystalline cellulose (NCC) is extracted as a colloidal suspension by acid hydrolysis of cellulosic materials, such as bacteria, cotton, and wood pulp. NCC has unique properties which make it an interesting starting point for the development of new materials. It is constituted of cellulose, a linear polymer of β(1→4) linked D-glucose units, the chains of which arrange themselves to form crystalline and amorphous domains. Colloidal suspensions of cellulose nanocrystals form chiral nematic structures upon reaching a critical concentration. The cholesteric structure consists of stacked planes of molecules aligned along a director (*n*), with the orientation of each director rotated about the perpendicular axis from one plane to the next. This structure forms spontaneously in solutions of rigid, rod-like molecules, and when the particles involved are optically active, chiral nematic structures may be formed.

1. Introduction

In order to reinvigorate the forestry sector and pulp manufacture for the 21st century, we must venture into *evolutionary developmental processes* aiming to re-engineer and custom design the industry's primary raw material, the lignocellulosic fibers, and profit from their unique properties. The competitiveness of forestry materials rests on associating product development with the concepts of fiber engineering and selective design, by using new technical tools to manipulate and re-structure fibers (and their constituents) at a scale as small as scientifically possible in order to add functionality. Functional materials based on lignocellulosics have the potential to compete with other established ones, e.g.

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

plastics, as well as metals and metal alloys, not only on performance, but also on merits of recyclability, biodiversity, biodegradability, sustainability and being a renewable resource.

Nanoscience, where physics, chemistry, biology and materials science converge, deals with the manipulation and characterization of matter at molecular to micron scale. Nanotechnology is the emerging engineering discipline that applies nanoscience to create products. Because of their size, nanomaterials have the ability to impart novel and/or significantly improved physical (strength, stiffness, abrasion, thermal), chemical (catalytic, ion-exchange, membranes), biological (anti-microbial, compatibility) and electronic (optical, electrical, magnetic) properties. While the chemistry and physics of simple atoms and molecules is fairly well understood, predictable and no longer considered overly complex, serious attempts to bridge across the length scales from nano to macro remain a major challenge, and will occupy researchers and scientists for years ahead.

The term nanotechnology embraces a broad range of science and technology working at a length scale of approximately 1 to 100 nanometers to manipulate atoms and molecules in order to create useful structures. The many successes that are currently attributed to nanotechnology have in fact been the result of years of research into conventional fields like materials or colloids sciences, and some of today's nanotechnology products are even mundane, when compared to (unchallenged) popular perceptions: stain-resistant trousers, better sun cream and tennis rackets reinforced with carbon nanotubes.

Lignocellulosic fibres are built from nanofibrils comprising a number of chain molecules in close alignment and lie parallel to one another (see Figure 1) with cross-sectional dimensions in the nanometer range (*1*). These nanofibrils comprise crystallites linked by amorphous areas (Figure 2a). A cellulose crystallite is essentially an aggregation of cellulose molecular chains of relatively low flexibility which tend to aggregate in parallel bundles with the desirable consequence of their axial physical properties approaching those of perfect crystals (*2*). The size, shape and orderliness of the crystallite regions play a predominant role as far as such fibre properties as tensile strength, density, rigidity, swelling and heat-sensitivity are concerned. The less ordered areas and the ratio of such amorphous areas to the crystalline regions are more influential in controlling extensibility.

One of the most important properties of cellulose, a linear, long-chain polymer of 1,4-linked, β-D-glucose, is the tendency to form fibrils and then fibres. The biosynthesis of cellulose in wood and plants proceeds by the progressive inclusion of glucose units into long, slender, monocrystalline fibrillar structures, within which the cellulose molecules are arranged parallel to one another with a twofold screw symmetry along their length (*3*). These fibrils consist of cellulose chains stabilized laterally by hydrogen bonds between the hydroxyl groups and oxygen of adjacent molecules (*4*). Depending on their origin, the diameter of the fibrils ranges from about 2 to 20 nm, and their lengths several tens of microns. As they are devoid of chain folding (*5*) and contain only a smaller number of defects (*6*), fibrils have a high elastic modulus close to that of a perfect cellulose crystal – estimated to be 150 GPa (*7*) – and a strength that should be on the order

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of 10 GPa. The structural defects that do exist allow the transverse cleavage of the fibrils into short nanocrystals, or nanofibrils, under acid hydrolysis.

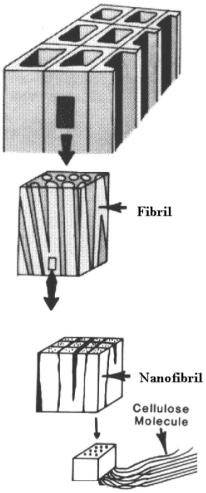
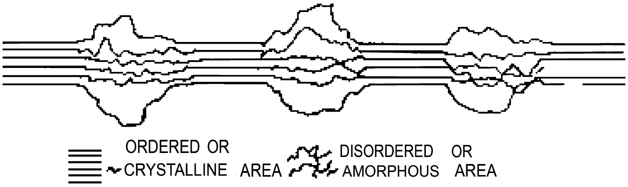
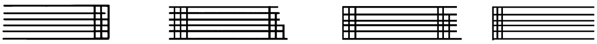

Fibril
Nanofibril
Cellulose Molecule

Figure 1. The ultrastructure of lignocellulosic fibres (1).


ORDERED OR CRYSTALLINE AREA
DISORDERED OR AMORPHOUS AREA

(a) Crystallites linked by amorphous areas



(b) Isolated crystallites

Figure 2. Schematic presentation of the crystalline structure of cellulose (adapted from ref (2)).

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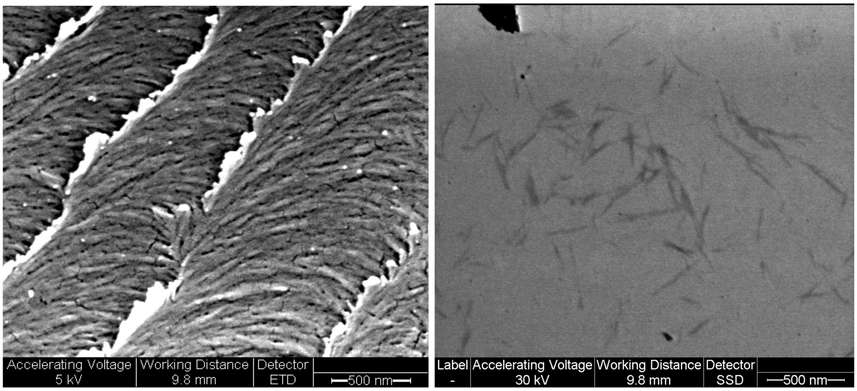

Figure 3. FE-SEM (left) and STEM (right) images of NCC film and suspension, respectively. The left image clearly depicts the chiral nematic orientation of the NCC crystals within each layer of dried NCC film. The image on the right illustrates the typical dimensions of NCC crystals in dilute water suspension. Both stubs were coated with AuPd and examined in high vacuum mode.

Extraction is central to further developing and processing cellulose nanocrystals into functional, high value-added materials, and as such several techniques are available. A reliable recipe for their production with uniform size, shape and charge distribution is necessary; however, it is challenging. The success of any approach would be measured by two essential factors: (1) The feasibility of cost-effective commercial scale-up, and (2) the manner in which yield of the cellulose nanocrystals may be maximized. Typically, two primary steps comprise the process of extracting cellulose nanofibrils from wood (*8*): (1) a controlled chemical pretreatment to destroy the molecular bonds whereby crystalline nanofibrils are hinged together in a network structure; and (2) appropriate use of mechanical energy to disperse substantial quantities of unhinged nanofibrils in the aqueous phase.

Employing acid hydrolysis, typically using concentrated mineral acids, such as sulfuric or hydrochloric, individual crystallites are prepared from wood pulp (*9*, *10*) or cotton (*11*, *12*) – each having a different extent of cellulose purity: cotton-seed fluff is composed of ≤ 94% cellulose, and wood contains ≤ 55%. The preparation procedure begins with initial acid action to remove the polysaccharide material closely bonded to the microfibrillar surface, resulting in an overall decrease in amorphous material. Subsequent hydrolysis breaks down those portions of the long glucose chains in accessible, non-crystalline regions. A levelling-off degree of polymerization ($90 \leq DP \leq 100$) is achieved: this corresponds to the residual highly crystalline regions of the original cellulose fibre. When this level is reached, hydrolysis is terminated by rapid dilution of the acid. A combination of centrifugation and extensive dialysis is then employed to fully remove the acid, and a brief sonication completes the process to disperse the individual particles of cellulose and yield an aqueous suspension, as shown in Figure 3. The cellulose rods that remain after this treatment are almost entirely crystalline (crystallinity ≥ 85%) and as such are termed *crystallites*.

The precise physical dimensions of the crystallites depend on several factors, including the source of the cellulose, the exact hydrolysis conditions, and ionic strength. Besides, complications in size heterogeneity are inevitable owing to the diffusion-controlled nature of the acid hydrolysis. Typical figures for crystallites derived from different species vary: (3 – 5) x 180 ± 75 nm for bleached softwood Kraft pulp (*13*), 7 x (100 – 300) nm for cotton (*12*), and 20 x (100 – 2000) nm for *Valonia* (*14*). The high axial (length to width) ratio of the rods is important for the determination of anisotropic phase formation (*9*, *12*).

2. Fundamentals of NCC Extraction

The production of colloidal suspensions of cellulose by sulfuric acid hydrolysis of cellulose fibres was first proposed by Rånby (*8*) over half a century ago. Rånby's work (*15*) suggested that cotton and wood cellulose were built up from micellar strings of uniform width (~7 nm), and could be set free by mechanical treatment such as ultrasonic waves. Hydrolysis was viewed as cutting the micellar strings into short fragments, or micelles, while retaining their width. Rånby proposed that, in cellulose synthesis, micelles rather than molecules may represent the primary structural elements. This theory was subsequently refined by Frey-Wyssling's experimental observations (*3*), which provided evidence that elementary microfibrils, separated (and joined) by paracrystalline cellulose, represent the basic building units (*16*).

Within the same decade, development of the acid degradation of cellulose by (*17*) led to the commercialization of microcrystalline cellulose (MCC). MCC is cellulose which has been degraded to a degree of polymerization where there is little further decrease (the levelled off DP). This degradation can be achieved by either mechanical disintegration or by the hydrolysis of purified cellulose after 15 minutes in 2.5 N HCl at 105 ± 1°C (*18*). Because of its useful characteristics, including zero toxicity, good hygroscopicity, chemical inactivity and reversible absorbency (*19*, *20*), MCC, derived from high-quality wood pulp, has found wide use in pharmaceuticals as a tablet excipient and in food production (owing to its properties as a stabilizer, texturizing agent and fat-replacer).

Hydrolysis with sulfuric acid can result in the introduction of sulfate esters at the surface of the cellulose crystallites, leading to added electrostatic stabilization of the suspensions; and, at sufficiently high concentrations of such suspensions, birefringent, ordered, liquid phases may be observed (*21*). Subsequent work on carefully sulfuric acid-hydrolyzed and purified samples demonstrated that a chiral, nematic-ordered phase formed above a critical concentration (*9*). The phase-forming ability of a cellulose crystallite suspension depends on the mineral acid chosen for the initial hydrolysis. Use of either sulfuric or phosphoric acid yields a chiral nematic phase, but hydrochloric acid will not because it cannot form the required ester at the crystallite surface. The viscous suspension from hydrochloric acid hydrolysis can form a birefringent, glassy phase after a "post-sulfation" treatment (*22*). Further, Nishiyama (*23*) showed that evaporation under shear of suspensions of cellulose crystallites (from the green alga *Cladophora* sp.) gave a film of highly oriented uniaxial structure.

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Using acid hydrolysis, suspensions of cellulose crystallites have been prepared from a variety of sources, including bacterial cellulose (*24*), microcrystalline cellulose (*25*), sugar beat primary wall cellulose (*26*, *27*), cotton (*12*)), tunicate cellulose (*28–38*), softwood pulp (*9*, *10*, *13*), hardwood pulp (eucalyptus) (*39*), and wheat straw (*40*, *41*). The precise physical dimensions of the crystallites depend on several factors, including the source of the cellulose, the exact hydrolysis conditions, and ionic strength of the suspension. Size heterogeneity is inevitable owing to the diffusion-controlled nature of the acid hydrolysis. Typical figures for crystallites derived from different species vary: (3 – 5) x 180 ± 75 nm for bleached softwood kraft pulp (*13*, *37*), 7 x (100 – 300) nm for cotton (*12*, *42*), and 20 x (100 – 2000) nm for *Valonia* (*14*), (5 – 10) nm x (100 nm – several μm) for bacterial cellulose (*24*), and (10 – 20) nm x (100 nm – several μm) for tunicate cellulose (*32*). The high axial (length to width) ratio of the crystallites is important for anisotropic phase formation (*9*, *23*).

2.1. Effects of Hydrolysis Conditions and Sulfation on NCC Yield

Sulfuric acid hydrolysis of kraft pulp fibres is a heterogeneous process involving the diffusion of the acid into the pulp fibres, the cleavage of the glycosidic bond in cellulose, the possible sulfation of cellulosic hydroxyl groups (i.e. conversion of cellulose-OH to cellulose-OSO_3H), and other side-reactions such as dehydration or oxidation of the cellulose. Hemicelluloses, if present in the pulp fibres, will also undergo similar reactions but at a faster rate because of their higher reactivity. As the hydrolysis proceeds, the degree of polymerization (DP) of the cellulose molecules is expected to decrease while the crystallinity of the extracted, H_2O-insoluble cellulose materials is expected to increase because of the accessibility and selective hydrolysis of the amorphous regions of the cellulose molecules. The yield of the extracted, H_2O-insoluble cellulose materials is also known to decrease. However, how the yield, one key factor in determining the economics of the hydrolysis process, is related to the extent of sulfation and/or the DP of the cellulose has not been studied or discussed in the literature.

Figure 4 shows the data of the percentage yield vs. DP of the extracted, H_2O-insoluble cellulose materials from the commercial, softwood (60% western red cedar and 40% mix of spruce, pine and fir or SPF) kraft pulp under various hydrolysis conditions (Hamad and Hu, 2010). At 16 wt.% acid concentration, the DP decreased significantly from ~680 to 180 as the hydrolysis temperature was raised from 45 to 85 °C, but the yield decreased only slightly from 93.7 to 91.5%. This result indicates that, at this acid concentration and over the temperature range studied, the hydrolysis of the cellulose molecules which likely occurred selectively on the amorphous regions did not proceed to the extent where the hydrolyzed cellulose molecules had sufficiently low DP and/or sufficiently high degree of sulfation to become soluble in the hydrolysis medium. A spruce sulphite dissolving pulp with a DP of 160 could be made completely H_2O-soluble at ≥0.20-0.25 sulfate subsitution (i.e., ≥ 20-25 sulfate groups per 100 anhydroglucose units) achieved via a 2-step silylation-sulfation procedure (*43*). The yield loss of ~6-8% for the hydrolysis with 16 wt.% acid was due likely to the preferential hydrolysis and dissolution of the hemicelluloses from the pulp.

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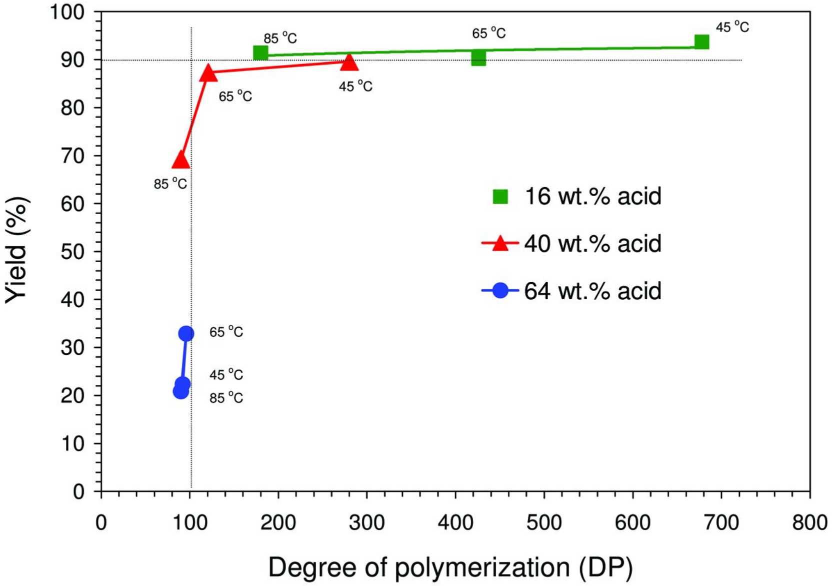

Figure 4. Yield (%) vs. DP of the extracted, H_2O-insoluble cellulose materials from the hydrolysis of the softwood kraft pulp (DP = 1178) at various sulfuric acid concentrations and temperatures for 25 min (after ref (10)).

At 40 wt.% acid concentration, the DP also decreased significantly from 280 to ~120 and the yield dropped only slightly when the temperature was increased from 45 to 65 °C (Figure 4). However, when the temperature was raised from 65 to 85°C, there was a relatively small decrease in DP from ~120 to 90 accompanied by a significant drop in the yield from 87.4 to 69.2%. This result indicates that when the more-difficult-to-hydrolyze, H_2O-insoluble, crystalline cellulose material reaches a DP of 90, the “easier-to-hydrolyze” amorphous cellulose material has a sufficiently low DP and/or high sulfation to dissolve. Because no sulfation was detected on the H_2O-insoluble material (Table I), the yield drop from 87.4 to 69.2% was likely due to lowered DP of the amorphous cellulose material at 40 wt.% acid concentration and 85 °C.

Interestingly, at 64 wt.% acid concentration, even though the DP values of the extracted cellulose materials (DP = 90-96) were similar to those of the extracted material from the hydrolysis at 40 wt.% acid concentration and 85 °C (DP = 90), the yields were much lower (20.9-32.9% vs. 69.2%).

It is possible that more sulfate ($-OSO_3H$) groups were introduced to (the surfaces of) both the crystalline and amorphous cellulose materials at 64 wt.% concentration than at 40 wt.% acid concentration. The greater the level of sulfation of the cellulose materials, the more H_2O-soluble the materials will become at similar DPs and the lower the yields of the extracted, H_2O-insoluble materials will be. Indeed, this was found to be the case by elemental analysis (Table I). Hydrolysis with 64 wt.% acid introduced 4.7-6.7 sulfate groups per 100 anhydroglucose units to the extracted, H_2O-insoluble cellulose materials, while hydrolysis with 40 wt.% acid did not introduce any detectable sulfate

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groups (Table I). These results illustrate that sulfation plays a significant role in determining the yield of the extracted, H_2O-insoluble cellulose materials within the 20 to 70% yield range.

2.2. Hydrolysis Reproducibility and Hydrolysis Time Effects

Table II lists the yields, elemental analysis data and DPs of the extracted cellulose materials for experiments on softwood kraft pulp using 64 wt.% sulfuric acid at 65 °C for 25, 15, and 5 minutes, respectively. Good reproducibility is evident for the yields of the extracted materials (c.v. = 1.8%), as well as elemental analysis (c.v. = 5.8%), for hydrolysis runs performed at 64 wt% acid and 65°C for 25 min. Decreasing the hydrolysis time from 25 to 15 min did not produce, statistically, any difference in the degree of sulfation or the yield. However, decreasing the hydrolysis time further to 5 min did give a slightly higher yield accompanied by a lower degree of sulfation (Table II). The coefficient of variation for all DP and intrinsic viscosity determinations are inherently higher than those for elemental analysis or yield but do not affect the interpretation of the results.

Table I. Elemental analysis data,[a] calculated sulfate group/anhydroglucose unit[b], yield and degree of polymerization (DP) of the extracted cellulose materials from hydrolysis of softwood kraft pulp at various sulfuric acid concentrations and temperatures for 25 minutes (*10*)

Acid (wt.%)	*Temp. (°C)*	*C (%)*	*H (%)*	*S (%)*	*-OSO_3H/ 100 glucose units (n)*	*Intrinsic viscosity $[\eta]$ (mL/g)*	*DP*	*Yield (%)*
-	-	42.16	5.83	<0.3	-	803.10	1179	100
16	45	41.97	5.77	<0.3	-	486.91	678	93.7
16	65	-	-	-	-	319.68	426	90.2
16	85	-	-	-	-	146.43	180	91.4
40	45	42.44	5.98	<0.3	-	218.90	280	89.6
40	65	42.33	6.09	<0.3	-	102.37[c]	121[c]	87.4[c]
40	85	42.31	6.13	<0.3	-	78.24	90	69.2
64	45	40.78	5.78	0.92	4.7	79.45	92	22.4
64	65	37.35	5.95	1.03	5.3	82.72	96	32.9
64	85	43.37	5.63	1.29	6.7	77.98	90	20.9

[a] Performed on freeze-dried samples, and N (%) $\leq$ 0.3 for all analysed samples. [b] n = 100 x 162.141 x S(%)/[3206.6 – 80.063 x S(%)], based on the formula of $C_6H_9O_4\text{-}(OH)_{1-n}\text{-}(OSO_3H)_n$ [i.e., $C_6H_{10}O_5\text{-}(SO_3)_n$] and calculated from the equation of 100 x 100 x n x S (%) / [6 x C + 10 x H + (5 + 3 x n) x O + n x S] = S (%) analyzed. [c] Average value from four hydrolysis experiments; DP = 121 (standard deviation, σ = 3) and yield = 87.4 % (σ = 0.86).

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Table II. Elemental analysis data,[a] calculated sulfate group/100 anhydroglucose units, yield and DP of the extracted cellulose materials from the hydrolysis of bleached softwood kraft pulp using 64 wt.% of sulfuric acid at 65°C for various times. Statistical values are indicated at the end of each segment: mean, ỹ, in italics, standard deviation, σ, in brackets, and the coefficient of variation, c.v. (%) = (σ/ỹ) x 100 (*10*)

Time (min)	*Run*	*C (%)*	*H (%)*	*S (%)*	*-SO_3H/100 glucose units (n)*	*Intrinsic viscosity [η] (mL/g)*	*DP*	*Yield (%)*
25	1	39.49	6.06	0.90	4.6	97.23	114	34.2
25	2	38.86	5.86	0.97	5.0	99.63	118	33.7
25	3	-	-	-	-	98.72	116	34.1
25[b]		37.35	5.95	1.03	5.3	82.72	96	32.9
ỹ					5.0	94.58	111	33.7
σ					(0.29)	(7.97)	(10)	(0.6)
c.v. (%)					5.8	8.43	9	1.8
15	1	-	-	-	-	110.48	132	36.0
15	2	39.44	5.95	0.92	4.8	92.78	109	33.4
15	3	-	-	-	-	84.80	98	33.5
ỹ						96.02	113	34.3
σ						(13.14)	(17)	(1.5)
c.v. (%)						13.68	15	4.4
5	1	-	-	-	-	115.32	138	38.7
5	2	39.73	5.98	0.76	3.9	86.89	101	39.1
5	3	-	-	-	-	84.68	98	37.7
ỹ						95.63	112	38.5
σ						(17.09)	(22)	(0.7)
c.v. (%)						17.87	20	1.8

[a] Performed on freeze-dried samples, N (%) ≤ 0.3 for all analyzed samples. [b] From previous experiment described in Table I.

In the literature, sulfation of the extracted, H_2O-insoluble cellulose materials from sulfuric acid hydrolysis is determined indirectly based on an increase in sulfur content, S (%), from elemental analysis and/or an increase of strong acid groups from conductivity titration (*11*, *12*, *24*). Araki (*13*) attempted to confirm the sulfation of an extracted cellulose material with a strong acid group content of 84 mmol/kg pulp (1.4 sulfate groups per 100 anhydroglucose units) by FT-IR, but reported that no absorbance due to sulfate groups at “1400-1600 and 1200 cm^{-1}” could be detected (*13*). Hamad and Hu (*10*) have carried out FT-IR spectral analysis on their extracted cellulose materials such as the ones with 4.7 sulfate groups per 100 anhydroglucose units from the 64 wt.% sulfuric acid hydrolysis at 45 °C for 25 min. No IR peaks at 1400-1600 cm^{-1}, 1200 cm^{-1} or 1130 cm^{-1} could be detected in their samples. The IR peak at 1130 cm^{-1} has been detected and assigned to the cellulose-O-SO_3/sub>Na bond in sulfated cellulose possessing 157 sulfate groups (at C5 and C2 positions) per 100 anhydroglucose units (*44*). It is possible that the degrees of sulfation in our samples as well as those in Araki’s sample

(<7 sulfate groups per 100 anhydroglucose units) are too low to allow detection by FT-IR. Techniques for direct, unambiguous determination of the sulfation of cellulose during sulfuric acid hydrolysis remain to be developed.

3. Solid-State, Structural, and Morphological Characteristics of NCC

Hydrolysis at 64 wt.% sulfuric acid produces iridescent nanocrystalline cellulose (NCC), with $90 \leq DP \leq 96$ and 4.7-6.7 sulfate groups per 100 anhydroglucose units (Table I), which self assembles (Figure 5). The scanning electron images of Figure 5 depict an agglomeration of parallelepiped rod-like structures, which possess cross-sections in the nanometer range, while their lengths are orders of magnitude larger resulting in high aspect ratios. It is evident that upon drying, via lyophilization (freeze drying) or evaporation, the NCC crystallites, whose size is <10 nm (Table III), self-assemble to form the unique meso-scale geometries of Figure 5. This behavior has been confirmed for a wide selection of NCC extracted from different raw materials and dried using different conditions. The precise mechanisms for this self-assembly merit a detailed examination, and we will endeavour to address this in future publications. Moreover, the iridescence of these NCC self-assemblies (upon controlled air drying) is typically characterized by the finger-print patterns shown in the optical image of Figure 5, where the patch work of bright and dark regions is typical of spherulitic behaviour of fibrillar crystals in which the molecules are packed with their axes perpendicular to the fibrillar axis. NCC is also characterized by high crystallinity (~80-90%) approaching the theoretical limit of the cellulose chains (Table III). However, hydrolysis at 16 or 40 wt.% sulfuric acid, produces partially hydrolyzed pulp (PHP) with crystallinity values ranging 18-27% over the starting pulp and DP ≥ 90, but without any detectable sulfate groups or iridescent behaviour (*10*).

The wide-angle X-ray (WAXD) diffraction patterns (Figure 6) of freeze-dried nanocrystalline cellulose consist of well-defined peaks assigned to the (002) plane at $2\theta \approx 22.7°$ and the (101) and $(10\overline{1})$ planes at $2\theta \approx 14\text{-}17°$, as well as a contribution for the (040) plane at $2\theta \approx 34.3°$. This agrees with published data (see ref (*45*) for instance) indicating cellulose I structure with a high degree of crystalline perfection. The WAXD patterns for hydrolysis at different temperatures vary in crystalline peak intensity and width – leading to different X_c and D – but are otherwise similar in overall response (Figure 8 and Table III). The raw diffraction patterns of the extracted materials are resolved, using the Ruland-Rietveld approach into their respective crystalline and amorphous regions based on known cellulose I peaks, and the solid-state properties presented in Table III are thus determined [The Ruland-Rietveld approach is based on the work of Rietveld (*46*), Ruland (*47*) and Vonk (*48*).]

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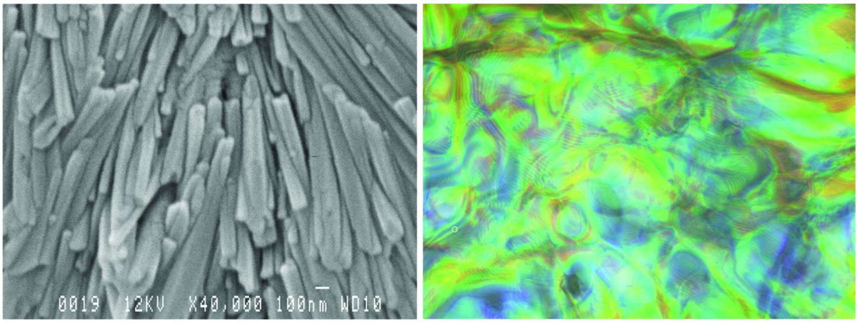

Figure 5. SEM image (left) of lyophilized NCC extracted from bleached softwood kraft pulp using 64% sulfuric acid at 45°C for 25 minutes. The morphology of the NCC self-assemblies is characterized by agglomerates of parallelepiped, rod-like structures of nano-scale cross-sections. The cross-polarized reflection optical image (right) of air-dried NCC suspension clearly shows the fingerprint local periodic structure (40X magnification).

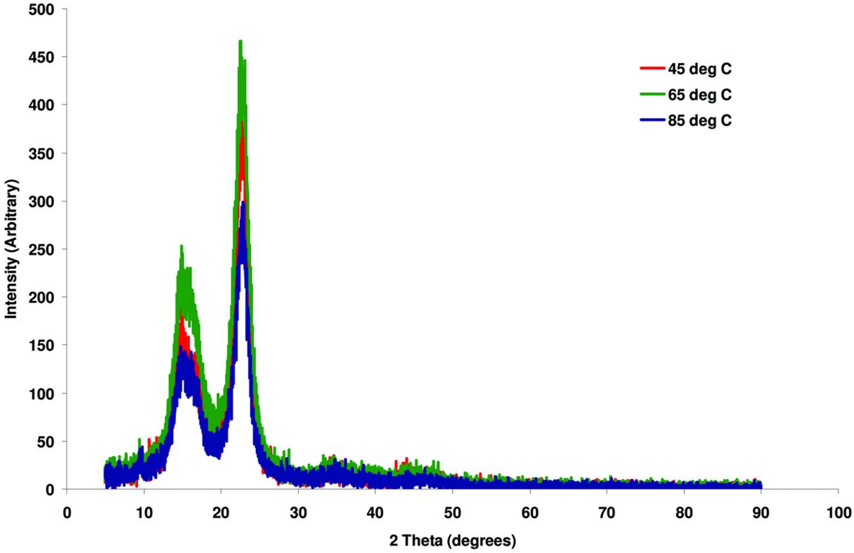

Figure 6. Wide-angle X-ray diffractograms of (lyophilized) NCC from bleached kraft softwood pulp using 64 wt.% sulfuric acid for 25 minutes at the indicated temperatures.

Table III. Solid-state attributes of the extracted cellulose materials based on X-ray diffractometric analysis. All sulphuric acid hydrolysis conditions were carried out for 25 minutes, unless otherwise noted.

Hydrolysis Conditions	*Crystallite Size D (nm)*	*Crystalline Area A_c*	*Amorphous Area A_a*	*Crystallinity X_c (%)*
16 wt.%, 45 °C	*16.8*	*23.0*	*10.4*	*68.9*
16 wt.%, 65 °C	*16.0*	*26.8*	*10.9*	*71.1*
16 wt.%, 85 °C	*14.0*	*28.8*	*9.9*	*74.4*
40 wt.%, 45 °C	*16.1*	*22.5*	*8.6*	*72.4*
40 wt.%, 65 °C	*11.4*	*45.5*	*16.6*	*73.2*
40 wt.%, 85 °C	*11.4*	*47.2*	*16.2*	*74.4*
64 wt.%, 45 °C	*8.3*	*37.9*	*4.6*	*89.1*
64 wt.%, 65 °C	*6.1*	*33.8*	*8.7*	*79.6*
64 wt.%, 65 °C, 15 min	*5.9*	*27.9*	*7.3*	*79.3*
64 wt.%, 65 °C, 5 min	*5.8*	*37.4*	*8.8*	*80.9*
64 wt.%, 85 °C	*7.1*	*26.7*	*7.0*	*79.3*
Pulp	*15.7*	*53.6*	*37.8*	*58.6*

It is revealing that the crystallite size is within a fairly consistent range (5.8 nm $\leq D \leq$ 8.3 nm) for NCC extracted using 64 wt.% for the range of temperatures and hydrolysis times indicated (Table III). The amorphous component of the X-ray diffraction pattern is also consistently low for NCC ($4.6 \leq A_a \leq 8.8$), confirming the high degree of order associated with these materials. Extraction using 64 wt% sulfuric acid hydrolysis at 45 °C for 25 minutes produced the highest crystallinity (~90%) – the closest attainable attribute to perfect cellulose chains [Repeated extractions at same conditions produced similar results.]. The WAXD analysis (outlined in Table III) indicates that the nano-rods of Figure 5 in essence consist of NCC crystallites (5.8 nm $\leq D \leq$ 8.3 nm)—cf. Figure 3. Hydrolysis at lower sulfuric acid concentrations (16 and 40 wt.%) reduced the amorphous component from the starting pulp; however, the conditions are inadequate to structurally re-align the crystallites or reduce the crystallite size. As a result, partially hydrolyzed pulps of higher crystallinity (68.9% $\leq X_c \leq$ 74.4%) are produced. The data indicate a different extent – and possibly mechanism – of scission of the crystalline cellulose chains occurs during lower-acid concentration hydrolysis.

In conclusion, there is a minimum DP threshold (~90-100) associated with the formation of nanocrystalline cellulose (NCC) of high crystallinity (> 80%) and (relatively) small crystallite size (≤ 8 nm). At high acid concentration (64 wt.% H_2SO_4), crystallinity reaches a maximum at around 90% while crystallite size decreases. Theoretically, a controlled chemical destruction of the (accessible) amorphous regions would translate into engineering cellulose materials of specified strength by controlling the ordered/crystalline regions and specified stretch by controlling the disordered/amorphous regions in the cellulose chains. Thus, while the 64 wt.% (45, 65, and 85 °C) and 40 wt.% (65 and 85 °C) share a statistically similar DP range (90 ≤ DP ≤ 121), the 64 wt.% cases (at all

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temperatures and hydrolysis times) are characterized by a smaller crystallite size (5.8 nm ≤ D ≤ 8.3 nm) and higher crystallinity (79.3% ≤ X_c ≤ 89.1%) than the 40 wt.% cases. The 64 wt.% cases are solely shown to have measurable sulfate groups per 100 anhydroglucose units (4.7-6.7) when compared to the 40 wt.% cases. These results show that sulfation is important in imparting the unique NCC characteristics to the extracted cellulose materials.

4. Optical Properties of NCC

When NCC is extracted from kraft wood pulp using sulfuric acid hydrolysis, the result is a suspension consisting of rods (also known as whiskers) that are ~5 nm in width and ~150 nm long that neither precipitate nor flocculate. The rods have an overall negative charge density, so that if they are present in dilute aqueous solution, they will form a single isotropic phase where the particles experience electrostatic repulsion. If the concentration of the suspension is increased (e.g. by removal of water), then phase separation occurs: the sample is divided into an isotropic phase on top, and an anisotropic phase at the bottom of the tube. In the latter phase, the proximity of the rods causes them to self-orient along a vector director, thereby resulting in the formation of a nematic liquid crystal. When the suspension reaches a critical concentration, the rods form a chiral nematic ordered phase, which displays characteristics typical of a cholesteric liquid crystal (i.e. rods organized in layers, with a director axis which varies from layer to layer in a periodic fashion) [It is important to note that the phase-forming ability of cellulose depends on the acid chosen for hydrolysis.]. Chiral nematic liquid crystals (i.e. ordered fluids) whose pitch is of the order of the wavelength of visible light reflect circularly polarized light of the same handedness as the chiral nematic phase (*49*). The wavelength of this selectively reflected light changes with viewing angle, leading to an iridescent appearance. While it has been well established that cellulose derivatives could form iridescent liquid and solid phases (*5*, *50–52*) showed that by simply casting films from suspensions of cellulose crystallites, cellulose films with the optical properties of chiral nematic liquid crystals could also be prepared.

Over the years, a number of studies have demonstrated how it is possible to perturb the chiral nematic phase in pure NCC, using salt (*42*), magnetic fields and shear alignment (*53*). The disruption of the cholesteric phase in NCC is believed to occur via the perturbation of the interactions between the rods, resulting in the formation of helically twisted rods (*53*, *54*). Recent studies have proposed refined structures of cellulose I (*55*, *56*). Putting these findings together, a structural model of NCC could be built at the molecular, rod and liquid crystal level (Figure 7). It then becomes possible to examine what happens to NCC at a molecular level (Figure 7a) as a function of ionic strength, temperature, suspension concentration, and exposure to magnetic field, and on the other, what happens to NCC at the chiral nematic phase level (Figure 7c), as a function of these same parameters.

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Effect of Ionic Strength

The ionic strength in a cellulose suspension can affect the formation of the anisotropic phase. Since the cellulose rods have an overall negative charge density, the sodium ion in NaCl interacts strongly with the surface of the NCC rods, thereby shielding the surface charges and reducing the effect of Coulombic repulsion. As described by Stroobants (*57*), in this case, twisting forces between polyelectrolytes will impede the ordering of the charged NCC rods. This will prevent the parallel alignment of the charged NCC rods, shifting the Onsager isotropic-anisotropic phase transition to higher concentrations. In other words, with increasing electrolyte concentration, the critical concentration required to spontaneously form the anisotropic phase increases, and consequently the pitch and the volume fraction of the anisotropic phase in the biphasic cellulose suspension decreases. Data by Pan (*56*) indicates that when the suspension is dried to form a film, this lower pitch is preserved, i.e. the drying process does not interfere with the interaction of the rods.

Pan (*56*) also suggested that the presence of sodium increases the propensity of the cellulose chains to become amorphous. In other words, the presence of salt possibly perturbs the intermolecular hydrogen bonds that form between O6 on one chain and O2/O3 on the other (Figure 7a). This perturbation is likely to be indirect and may arise from the formation of helically twisted rod (Figure 7b) with increased ionic strength. A twist in the rods would require a twist in the cellulose chains, around the C4-O1-C1 bond. Since both the C4 and C1 signals display an increased "amorphous" signal with increased NaCl concentration, it is possible that the twist would destroy the optimal packing between the cellulose chains and therefore weaken the intermolecular hydrogen bonds.

Effect of Temperature

A number of studies on derivatives of cellulose (e.g. (hydroxypropyl)cellulose, (2-Ethoxypropyl)cellulose, and (acetyl)(ethyl)cellulose) have shown that the pitch of the chiral nematic phase is highly dependent on temperature. For (hydroxypropyl)cellulose and (2-Ethoxypropyl)cellulose, it was found that P increases with increased temperature, whereas for (acetyl)(ethyl)cellulose samples with low degrees of acetyl substitution, P decreases with increased temperature, as observed here for our NCC samples. It has been shown that the type and degree of substitution on the cellulose chains affects the critical concentration required for the formation of the chiral nematic ordered phase and the temperature at which thermotropic phases exist. So presumably, in the case of (acetyl)(ethyl)cellulose with low degrees of acetyl substitution, on the one hand, and NCC, on the other, the interaction between the rods is weaker, thereby requiring a higher critical concentration to spontaneously form the anisotropic phase. As mentioned above, an increased critical concentration signifies a decrease in the pitch and the volume fraction of the anisotropic phase in the biphasic cellulose suspension. The interaction between the rods could be weakened by either unfavorable interactions between the substituents on the cellulose or the flexibility of the rods.

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If the former were true, then one would expect rods made from cellulose with the less bulky acetyl and ethyl groups (or in the case of NCC, no substituents) to favor rod-rod interactions and packing. Since this is not the case, then flexibility must play a role and the observation that higher temperatures (i.e. more flexibility) result in lower pitch are validated.

Effect of Suspension Concentration

The phenomenological observations of Uematsu and Uematsu (*58*) have led to a relationship of pitch in the cholesteric phase as a function of temperature and concentration. Empirical data reported by Pan (*56*) agree with the aforesaid. It was found that as the suspension concentration increases, so does the pitch. This agrees with other findings which suggest that an increased suspension concentration promotes the formation of proportionately more anisotropic phase. As the volume fraction of the anisotropic phase increases, then so does the pitch of the chiral nematic phase. Furthermore, Pan (*56*) showed that crystallinity increases as a function of concentration, suggesting that higher suspension concentrations favor better chain-chain interactions, leading to a better packing of the rods, leading in turn to more anisotropic phase. If the rods interact strongly as straight rods, then in the model shown in Figure 7, the cellulose chains adopt optimal packing (Figure 7a), leading to fewer amorphous domains.

Effect of Magnetic Field

A number of studies have shown that drying suspensions of NCC in a magnetic field results in a marked alignment with their long axis perpendicular to the direction of the field. Pan, et al. (*56*) have demonstrated that the presence of a magnetic field also has the effect of increasing the pitch of the chiral nematic phase, and that the pitch increases with increasing exposure time. Indeed, for a 0.5 % w/w NCC suspension, the pitch goes from 232 nm outside the magnetic field to 311 nm for suspensions exposed to the magnetic field. Likewise, the pitch in a 2.0 % w/w NCC suspension varies from 317 nm outside the magnetic to 371 nm for suspension exposed to a magnetic field. The magnetic field, however, does not seem to help promote chiral nematic phase formation in suspensions of low concentrations (0.1-0.2 % w/w). These observations suggest that the magnetic field does not influence the formation of chiral nematic structure, but only has an effect on the pitch of the resulting films. The increase in chiral nematic pitch may be due to the magnetic alignment of cellulose crystallites, given their anisotropic shapes and non-zero magnetic susceptibilities. This might cause the tilt angle between the directions of directors in adjacent pseudo-layers to decrease. Similar effects have been observed for DNA and other liquid crystals.

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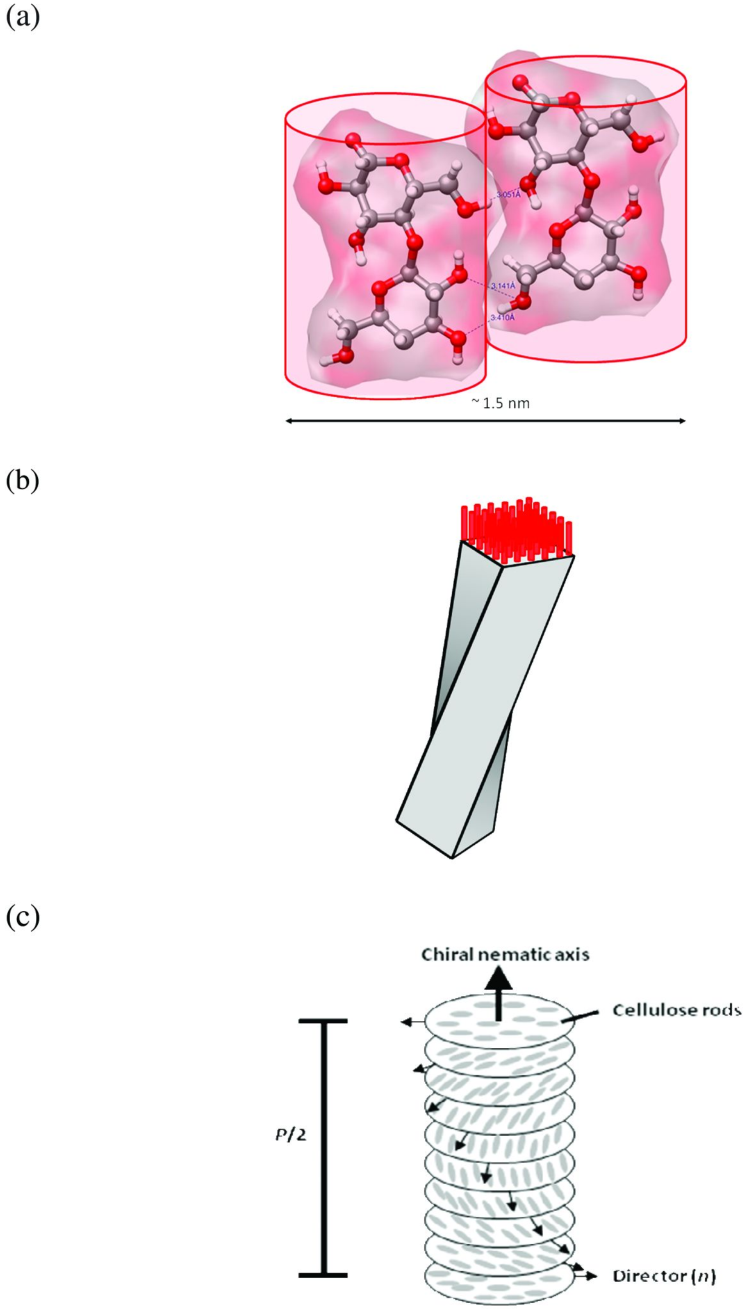
(a)
3.051Å
3.141Å
3.410Å
~1.5 nm
(b)
(c)
Chiral nematic axis
Cellulose rods
P/2
Director (n)

Figure 7. The three levels of structure in NCC films: (a) Model of two cellulose I chains in crystalline domains, based on the findings of Witter (55). The red cylinders represent the cellulose chains. (b) Assuming that the rods which come together to form the chiral nematic phase are 3 x 3 nm, then roughly nine of the

cellulose I chains, shown in red, would form a rod (grey). As reported by Orts (53) and by Lima and Borsali (54), these rods are helically twisted along their length of 20-200 nm, in the presence of high salt, for example; (c) The (straight or helically twisted) rods come together in layers to form the chiral nematic phase of NCC. (Adapted from ref (56))

5. Concluding Summary

Lignocellulosic fibres consist of concentric composite layers of cellulose fibrils embedded into an amorphous matrix of hemicellulose and lignin. Cellulose in plants is biosynthesized to form long, slender monocrystalline fibrils, within which the cellulose molecules are arranged parallel to one another with a twofold screw symmetry along their length. The cellulose chains in the fibrils are bundled laterally by intra- and inter-molecular hydrogen bonds. Hydrolytic processes can be used to hydrolyse the hemicellulose and amorphous cellulose leaving highly crystalline cellulose nanocrystals, also referred to as nanocrystalline cellulose (NCC). As these cellulose nanocrystals are very pure and have few defects, they have a high elastic modulus close to the theoretical value of a perfect cellulose crystal, which is 150 GPa. Because of its nano-size, NCC has a large surface area (~ 250 m^2/g), and has conductive properties. It also has a chiral nematic structure rendering NCC capable of producing unique optical properties, and has high strength approaching that of pure cellulose owing to NCC's high degree of crystallinity. NCC has a high potential to act as high-performance reinforcement in polymeric composites, thereby creating opportunities to develop functional, high-tenacity and highly-durable engineering materials from a renewable resource suitable for consumer, structural and medical applications.

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References

1. Frey-Wyssling, A.; Mühlethaler, K. *Ultrastructure Plant Cytology*; Elsevier, 1965, pp 34−40.
2. Battista, O. A. *Microcrystal Polymer Science*; McGraw-Hill, 1975.
3. Frey-Wyssling, A. The fine structure of cellulose microfibrils. *Science* **1954**, *119*, 80–82.
4. Phillipp, H. J.; Nelson, M. L.; Ziifle, H. M. Crystallinity of cellulose fibers as determined by acid hydrolysis. *Text. Res. J.* **1947**, *18*, 585–596.
5. McIntyre, J. E. Liquid Crystalline Polymers. In *Structure and Properties of Oriented Polymers*, 2nd ed.; Ward, I. M., Ed.; Chapman & Hall, 1997; pp 447−514.
6. Koukios, E. G.; Valkanas, G. N. Process for chemical separation of the three main components of lignocellulosic biomass. *Ind. Eng. Chem. Prod. Res. Dev.* **1982**, *21* (2), 309–314.
7. Sakurada, I.; Nukushina, Y. Experimental determination of the elastic modulus of crystalline regions in oriented polymers. *J. Polym. Sci.* **1962**, *57*, 651–660.

8. Rånby, B. G. The colloidal properties of cellulose micelles. *Discuss. Faraday Soc.* **1951**, *11*, 158–164.
9. Revol, J.-F.; Bradford, H.; Giasson, J.; Marchessault, R. H.; Gray, D. G. Helicoidal self-ordering of cellulose microfibrils in aqueous suspension. *Int. J. Biol. Macromol.* **1992**, *14*, 170–172.
10. Hamad, W. Y.; Hu, T. Q. Structure-process-yield interrelations in nanocrystalline cellulose extraction. *Can. J. Chem. Eng.* **2010**, *88*, 392–402.
11. Revol, J.-F.; Godbout, L.; Dong, X. M.; Gray, D. G.; Chanzy, H.; Marel, G. Chiral nematic suspensions of cellulose crystallites; phase separation and magnetic field orientation. *Liq. Cryst.* **1994**, *16* (1), 127–134.
12. Dong, X. M.; Revol, J.-F.; Gray, D. G. Effects of microcrystallite preparation conditions on the formation of colloid crystals of cellulose. *Cellulose* **1998**, *5*, 19–32.
13. Araki, J.; Wada, M.; Kuga, S.; Okano, T. Flow properties of microcrystalline cellulose suspension prepared by acid treatment of native cellulose. *Colloids Surf., A* **1998**, *142*, 75–82.
14. Imai, T.; Boisset, C.; Samejima, M.; Igarashi, K.; Sugiyama, J. Unidirectional processive action of cellobiohydrolase Cel7A on *Valonia* cellulose microcrystals. *Fed. Eur. Biochem. Soc. Lett.* **1998**, *432*, 113–116.
15. Rånby, B. G. The cellulose micelles. *Tappi* **1952**, *35* (2), 53–58.
16. Based on the classical two-phase, fringed-micelle hypothesis, which basically considered fibrous polymers to be a mixture of ordered regions, or "crystallites," embedded in an amorphous matrix, chemical reactions of cellulose – as in acid hydrolysis – were interpreted to occur with the hydroxyl groups of chain segments in the amorphous regions. X-ray diffractometric studies reported another view whereby most (~95%) of the cellulosic material was thought to be a single, continuous phase exhibiting a spectrum of order, and the remaining (5%) part being the truly amorphous material in cellulose (*59*). According to this view, cellulose comprised aggregates of paracrystalline fibrils, and a low crystallinity value of native cellulose could be explained in terms of a paracrystalline molecular arrangement within the elementary fibrils.
17. Battista, O. A. Hydrolysis and crystallization of cellulose. *Ind. Eng. Chem.* **1950**, *42*, 502–507.
18. Batttista, O. A.; Coppick, S.; Howsmon, J. A.; Morehead, F. F.; Sisson, W. A. Level-off degree of polymerization – relation to polyphase structure of cellulose fibres. *Ind. Eng. Chem.* **1956**, *48*, 333–335.
19. Ardizzone, S.; Dioguardi, F. S.; Mussini, T.; Mussini, P. R.; Rondinini, S.; Vercelli, B.; Vertova, A. Microcrystalline cellulose powders: Structure, surface features and water sorption capability. *Cellulose* **1999**, *6* (1), 57–69.
20. Battistia, O. A.; Smith, P. A. Level-off D.P. Cellulose Products. U.S. Patent 2,978,446, 1961.
21. Marchessault, R. H.; Morehead, F. F.; Walter, N. M. Liquid crystal systems from fibrillar polysaccharides. *Nature* **1959**August22, *184*, 632–3.
22. Araki, J.; Wada, M.; Kuga, S.; Okano, T. Birefringent glassy phase of a cellulose microcrystal suspension. *Langmuir* **2000**, *16* (6), 2413–2415.

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23. Nishiyama, Y.; Kuga, S.; Wada, M.; Okano, T. Cellulose microcrystal film of high uniaxial orientation. *Macromolecules* **1997**, *30* (20), 6395–6397.
24. Roman, M.; Winter, W. T. Effect of sulfate groups from sulfuric acid hydrolysis on the thermal degradation behavior of bacterial cellulose. *Biomacromolecules* **2004**, *5*, 1671–1677.
25. Araki, J.; Wada, M.; Kuga, S.; Okano, T. Influence of surface charge on viscosity behavior of cellulose microcrystal suspension. *J. Wood Sci.* **1999**, *45*, 258–261.
26. Dinand, E.; Chanzy, H.; Vignon, M. R. Suspensions of cellulose microfibils from sugar beet pulp. *Food Hydrocolloids* **1999**, *13*, 275–283.
27. Dufresne, A.; Cavaille, J. Y.; Vignon, M. R. Mechanical behavior of sheets prepared from sugar beet cellulose microfibrils. *J. of Appl. Polymer Sci.* **1997a**, *64*, 1185–1194.
28. Azizi Samir, M. A. S.; Alloin, F.; Gorecki, W.; Sanchez, J.-Y.; Dufresne, A. Nanocomposite polymer electrolytes based on poly(oxyethylene) and cellulose nanocrystals. *J. Phys. Chem. B* **2004a**, *108* (30), 10845–10852.
29. Azizi Samir, M. A. S.; Alloin, F.; Paillet, M.; Dufresne, A. Tangling effect in fibrillated cellulose reinforced nanocomposites. *Macromolecules* **2004b**, *37* (11), 4313–4316.
30. Cavaille, J.; Chanzy, H.; Fleury, E.; Sassi, J. Surface-Modified Cellulose Microfibrils, Method for Making the Same, and Use Thereof as a Filler in Composite Material. U.S. Patent 6,117,545, 2000a.
31. Cavaille, J.; Chanzy, H.; Favier, V.; Ernst, B. Cellulose Microfibril-Reinforced Polymers and Their Applications. U.S. Patent 6,103,790, 2000b.
32. Favier, V.; Chanzy, H.; Cavaille, J. Y. Polymer nanocomposites reinforced by cellulose whiskers. *Marcomolecules* **1995a**, *28*, 6365–6367.
33. Favier, V.; Canova, R.; Cavaille, J.; Chanzy, H.; Dufresne, A.; Gauthier, C. Nanocomposite materials from latex and cellulose whiskers. *Polym. Adv. Technol.* **1995b**, *6*, 351–355.
34. Hajji, P.; Cavaille, J. Y.; Favier, V.; Gauthier, C.; Vigier, G. Tensile behavior of nanocomposies from latex and cellulose whiskers. *Polym. Compos.* **1996**, *17* (4), 612–619.
35. Heux, L.; Dinand, E.; Vignon, M. R. Structural aspects in ultrathin cellulose microfibrils followed by ^{13}C CP-MAS NMR. *Carbohydr. Polym.* **1999**, *40*, 115–124.
36. Heux, L.; Chauve, G.; Bonini, C. Nonflocculating and chiral-nematic self-ordering of cellulose microcrystals suspensions in nonpolar solvents. *Langmuir* **2000**, *16* (21), 8210–8212.
37. Orts, W. J.; Shey, J.; Imam, S. H.; Glenn, G. M.; Gutman, M. E.; Revol, J.-F. Application of cellulose microfibrils in polymer nanocomposites. *J. Polym. Environ.* **2005**, *13* (4), 301–306.
38. Schroers, M.; Kokil, A.; Weder, C. Solid polymer electrolytes based on nanocomposites of ethylene oxide-epichlorohydrin copolymers and cellulose whiskers. *J. Appl. Polym. Sci.* **2004**, *93*, 2883–2888.
39. Beck-Candanedo, S.; Roman, M.; Gray, D. Effect of reactions conditions on the properties and behavior of wood cellulose nanocrystal suspensions. *Biomacromolecules* **2005**, *6*, 1048–1054.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch012

40. Dufresne, A.; Cavaille, J. Y.; Helbert, W. Thermoplastic nanocomposites filled with wheat straw cellulose whiskers. Part II: Effect of processing and modeling. *Polym. Compos.* **1997b**, *18* (2), 198–210.
41. Helbert, W.; Cavaille, J. Y.; Dufresne, A. Thermoplastic nanocomposites filled with wheat straw cellulose whiskers. Part I: Processing and mechanical behavior. *Polym. Compos.* **1996**, *17* (4), 604–611.
42. Dong, X. M.; Kimura, T.; Revol, J.-F.; Gray, D. G. Effects of ionic strength on the isotropic−chiral nematic phase transition of suspensions of cellulose crystallites. *Langmuir* **1996**, *12* (8), 2076–2082.
43. Wagenknecht, W.; Nehls, I.; Klemm, D.; Philipp, B. Synthesis and substitution distribution of Na-cellulose sulfates via O-trimethylsilyl cellulose as intermediate. *Acta Polym.* **1992**, *43*, 266–269.
44. Baumann, H.; Richter, A.; Klemm, D.; Faust, V. Concepts for preparation of novel regioselective modified cellulose derivatives sulfated, aminated, carboxylated and acetylated for hemocompatible ultrathin coatings on biomaterials. *Macromol. Chem. Phys.* **2000**, *201* (15), 1950–1962.
45. Wada, M.; Okano, T.; Sugiyama, J. Synchrontron-radiated X-ray and neutron diffraction study of native cellulose. *Cellulose* **1997**, *4*, 221–232.
46. Rietveld, H. M. A profile refinement method for nuclear and magnetic structures. *J. Appl. Cryst.* **1969**, *2*, 65–71.
47. Ruland, W. X-ray determination of crystallinity and diffuse disorder scattering. *Acta Cryst.* **1961**, *14*, 1180–1185.
48. Vonk, C. G. Computerization of Ruland's X-ray method for determination of the crystallinity in polymers. *J. Appl. Cryst.* **1973**, *6*, 148–152.
49. De Vries, H. L. Rotary power and other optical properties of certain liquid crystals. *Acta Crystallogr.* **1951**, *4*, 219–226.
50. Chandrasekhar, S. *Liquid Crystals*, 2nd ed.; Cambridge University Press, 1992.
51. Revol, J.-F.; Godbout, D. L.; Gray, D. G. Solidified Liquid Crystals of Cellulose with Optically Variable Properties. U.S. Patent 5,629,055, 1997.
52. Revol, J.-F.; Godbout, L.; Gray, D. G. Solid self-assembled films of cellulose with chiral nematic order and optically variable properties. *J. Pulp Pap. Sci.* **1998**, *24* (5), 146–149.
53. Orts, W. J.; Godbout, L.; Marchessault, R. H.; Revol, J.-F. Enhanced ordering of liquid crystalline suspensions of cellulose microfibrils: A small angle neutron scattering study. *Macromolecules* **1998**, *31* (17), 5717–5725.
54. Lima, M. M. D.; Borsali, R. Rod-like cellulose microcrystals : Structure, properties and applications. *Macromol. Rapid Commun.* **2004**, *25* (7), 771–787.
55. Witter, R.; Sternberg, U.; Hesse, S.; Kondo, T.; Koch, F. T.; Ulrich, A. S. C-13 chemical shift constrained crystal structure refinement of cellulose I-alpha and its verification by NMR anisotropy experiments. *Macromolecules* **2006**, *39* (18), 6125–6132.
56. Pan, J.; Hamad, W.; Straus, S. K. Parameters affecting the chiral nematic phase of nanocrystalline cellulose films. *Macromolecules* **2010**, *43*, 3851–3858.

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57. Stroobants, A.; Lekkerkerker, H. N. W.; Odijk, T. Effect of electrostatic interaction on the liquid-crystal phase-transition in solutions of rodlike polyelectrolytes. *Macromolecules* **1986**, *19* (8), 2232–2238.
58. Uematsu, Y.; Uematsu, I. Structure and Properties of Polyglutamates in Concentrated Solutions. In *Mesomorphic Order in Polymers* Blumstein, A.; ACS Symposium Series 74; American Chemical Association: Washington, DC, 1978; pp 136−156.
59. Bonart, R.; Hosemann, R.; Motzkus, F.; Ruck, H. X-ray determination of crystallinity in high-polymeric substances. *Norelco-Rep.* **1960**, *7* (3), 81.

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Chapter 13

Biofibers

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We depend on our forests and agricultural lands to supply most of our biofiber needs. For pulp and paper, we depend on a sustainable wood resource while turning to nonwood agricultural biomass for alternative supply. For building materials, such as biofiber boards, we also depend on wood however many countries are running out of wood and are beginning to depend more and more on biofibers from nonwood resources. While wood is the largest single source of biofiber in the world, nonwood agricultural biomass including bast, leaf and seed also contain biofibers that can be utilized. Biofibers can be used to produce a wide variety of products including biofiber boards (both flat and three dimensional, with and without an added adhesive, low to high density), molded biofiber (with and without added adhesives) for non-structural applications, biofiber nonwoven for filters and sorbents, and biofiber-reinforced composites for automobile and construction applications. Since biofibers are degradable by microorganisms, swell and shrink with changing moisture contents, combust and are degraded by ultraviolet radiation, we can modify the fibers to improve performance and increase the service life of the biofiber composites.

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

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Introduction

Biofibers

Biofibers in this chapter refer to lignocellulose and cellulose isolated from plant biomass, cellulose produced by bacteria and by tunicates (sea squirts) and regenerated cellulose that is chemically regenerated from natural cellulose. Hair, feather, wool and silk fibers are also biofibers however these will not be covered in this chapter.

Lignocellulose

a. Source of Supply

Lignocellulose is the most abundant renewable feedstock with about 200 billion tons produced annually (*1*), and it can be found in all plant biomass. In accordance with botanical classification, plant biomass can be grouped into six types as follows.

Type 1: Bast – jute, flax, hemp, ramie, kenaf, mesta, roselle
Type 2: Leaf – banana, sisal, pineapple, henequen, agave
Type 3: Seed – coir, cotton, kapok; coconut coir, oil palm empty fruit, bunches, rice, wheat, oat, rye
Type 4: Core – kenaf, hemp, jute, flax
Type 5: Grass – wheat, oat, barley, bamboo, corn, rice, bagasse
Type 6: Other – wood, roots

There are two general classes of plant biomass producing lignocellulose: primary and secondary. Primary plant biomass are those grown for their lignocellulose content while secondary plant biomass are those where the lignocellulose comes as a by-product from other primary utilization. Jute, hemp, kenaf, sisal, and cotton are examples of primary plant biomass while pineapple leaf (PALF), cereal stalks, agave, oil palm empty fruit bunches (EFB) and coconut coir (coir) are examples of secondary plant biomass. Some plant biomass contains more than one type of lignocellulose. For example, jute, flax, hemp, and kenaf have both bast and core type of lignocellulose. Lignocellulose may be of wood and non-wood origin where wood lignocellulose is subdivided into softwood and hardwood type, and non-wood lignocellulose is subdivided into bast, leaf, seed, core and grass type.

Table I shows an inventory of some of the world plant biomass now produced. The data for this table are extracted from several sources using estimates and extrapolations for some of the numbers. For this reason, the data should be considered to be only an estimated quantity of world plant biomass. The inventory of agricultural biomass production can be found in the FAO database on its web site. By using a harvest index, it is possible to estimate the quantity of harvest residue associated with a given production of a crop.

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Table I. An inventory of some of the world plant biomass

Source of Lignocellulose	*Dry weight (tone)*
Wood	1,750,000,000
Corn stalks	750,000,000
Wheat straw	600,000,000
Rice straw	360,000,000
Sorghum stalks	252,000,000
Barley straw	195,000,000
Sugarcane bagasse	102,200,000
Cotton stalks	68,000,000
Oil palm (Fronds + EFB)	57,000,000
Oat straw	55,000,000
Rye straw	40,000,000
Reeds	30,000,000
Bamboo	30,000,000
Cotton staple	18,300,000
Stem fibers (Kenaf, Jute)	13,700,000
Papyrus	5,000,000
Grass seed straw	3,000,000
Flax (oil seed)	2,000,000
Cotton linters	2,700,000
Leaf fibers (sisal, henneguen, maguey)	500,000
Esparto grass	500,000
Sabai grass	200,000
Hemp fibers	200,000
Abaca	80,000
Total	4,335,380,000

b. Structure and Composition

Depending on the type of plant biomass, plant parts and growth conditions, the structure and composition of lignocellulose vary greatly. Lignocellulose is a natural biocomposite of three major constituents, cellulose, hemicelluloses and lignin (*2*, *3*). The structure of lignocellulose is illustrated in Figure 1 and it can be described as cellulose-cemented in a matrix of lignin coupled by hemicelluloses.

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
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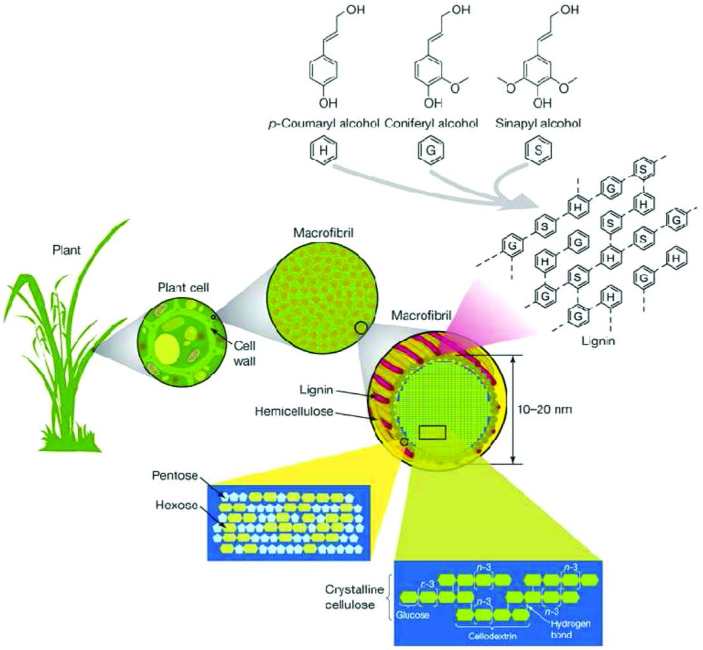
OH
OH
OH
OH
OH
OH
p-Coumaryl alcohol
Coniferyl alcohol
Sinapyl alcohol
H
G
S
Plant
Plant cell
Cell wall
Macrofibril
Macrofibril
Lignin
Lignin
Hemicellulose
10–20 nm
Pentose
Hexose
Crystalline cellulose
Glucose
n-3
Cellodextrin
Hydrogen bond

Figure 1. Illustration of lignocellusic structure. (Reproduced with kind permission (6) and Copyright of Nature)

Cellulose is the most abundant organic polymer on earth, and its characteristics shall be reviewed separately below. Lignin is the second most abundant biopolymer on earth next to cellulose. It is commonly believed that lignin is an amorphous copolymer of phenyl-propene units formed through random radical copolymerization of coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (*4*). Lignin is hydrophobic and it dissolves in alkaline but does not dissolve in most of organic solvents. It has high carbon and low hydrogen composition, and contains hydroxyl, methoxyl, ethylenic as its main functional groups (*5*). Lignin can be further polymerized via radical condensation. It is the matrix component of lignocellulose, and serves to glue cellulose, hemicelluloses and other cell wall components together. Generally, the higher the lignin content the harder the plant biomass. Lignin is responsible for the UV degradation of lignocellulose.

Hemicelluloses are amorphous and hydrophilic hetero-polysaccharides of C5- and C6-sugars. The polysaccharide chains of hemicelluloses are highly branched. The degree of polymerization of hemicelluloses is around 300-500. Hemicelluloses can be easily hydrolyzed by dilute acids, alkaline and selected enzymes. They contain hydroxyl and acetyl functional groups, and are responsible for the biodegradation of lignocellulose.

Table II. Chemical compositions of selected lignocellulose (*9–14*)

	Cellulose %	*Lignin* %	*Hemicelluloses* %	*Ash* %
Softwood	40-45	26-34	25-30	0.2-0.8
Hardwood	45-50	22-30	21-36	0.2-0.8
Cotton	85-90	0.7-1.6	5.7	0.8-2
Wheat straw	48.8	17.1	15-31	4-9
Rice straw	41-57	8-19	33	15-20
Rice husk	35-45	20	19-25	14-17
Ramie	68.6-76.2	0.6-0.7	13.1-16.7	-
Hemp	70.2-74.4	3.7-5.7	17.9-22.4	0.8
Flax	64-71	2-5	18.6-20.6	5
Kenaf	31-39	15-19	21.5-23	2-5
Jute	61-71.5	12-13	13.6-20.4	0.5-2
Abaca	56-63	7-9	15-17	3
Sisal	67-78	8-11	10-14.2	0.6-1
PALF	70-82	5-12	-	0.7-0.9
Henequen	77.6	13.1	4-8	-
Coir	36-43	41-45	0.15-0.25	2.7-10.2
Oil palm EFB	37-42	20-21	24-27	3.5

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Lignocellulose are valued for its intrinsic attributes (i) biodegradability, (ii) abundance with diversified sources of supply, (iii) low density, (iv) high specific strength (v) high stiffness (vi) non-abrasiveness (vii) good thermal properties (viii) polar and hydrophilic surface. These attributes are the result of its compositions and hierarchical structure (*7*). On the other hand, lignocellulose is disadvantaged in commercial applications by its (i) hydrophilic surface (ii) low processing temperature (<200 °C) (iii) potential to emit volatile cell wall components during processing (*8*). The chemical compositions and dimensions of selected lignocellulose are shown in Table II and Table III.

Table III. Dimensions of selected lignocellulose (*10*, *15*)

	Length *mm*	*Diameter* *μm*	*Density* *g/cm³*
Softwood	3.5-5	2-5	0.9-1
Hardwood	1-1.8	3	0.9-1
Cotton	-	20-50	1.4-1.6
Wheat straw	0.5-2.5	8-34	-
Rice straw	1.53	4-16	-
Rice husk	1.21	19	-
Hemp	5-55	10-51	1.48
Flax	9-70	5-38	1.5
Kenaf (Bast)	2-6	14-33	-
Jute	2-5	10-25	1.3
Sisal	1-8	8-41	1.5
Bagasse	0.8-2.8	10-34	-
PALF	62	20-80	0.8-1.6
Coir	1	10-20	1.2
Ramie	60-250	50	1.5
Oil palm EFB	0.48-0.53	150-500	0.7-1.55

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Cellulose

Cellulose is the major cell wall component of all plant lignocellulose (*16*). It is a linear mono-polysaccharide of glucose units linked head to tail with a chemical formula of $(C_6H_{10}O_5)_n$. In the cell wall of lignocellulose, cellulose is found largely in crystalline forms with varying small amount of amorphous cellulose.

Crystalline cellulose chains are stabilized by a strong and complex intra- and inter-molecular hydrogen bond network. The hydrogen-bond network and molecular orientation among crystalline cellulose can vary widely giving rise to six inter-convertible polymorphs of cellulose, namely, Cellulose I, II, III_I, III_{II}, IV_I, IV_{II}. Native cellulose of almost all origins is believed to be in cellulose I which contains two crystalline forms I_α and I_β at varying proportions (I_α: triclinic $P1$ unit cell a=6.717Å, b=5.962Å, c=10.400Å, α=118.08°, β=114.80° and γ=80.37°; I_β: monoclinic $P2_1$ unit cell a=7.784Å, b=8.201Å, c=10.38Å, α=β=90° and γ=96.5°) (*17–19*). The crystal structure of a glucose unit of cellulose is shown in Figure 2. The stacking pattern of linear polysaccharide chains in two crystalline forms I_α, I_β are shown in Figure 3.

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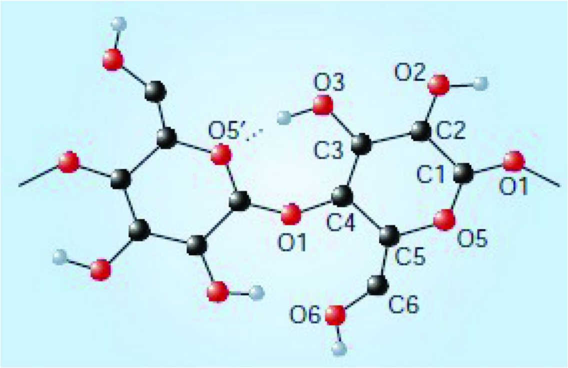

Figure 2. Structural unit of cellulose showing intra-molecular hydrogen bonds. (Reproduced with kind permission (19) and Copyright of Nature)

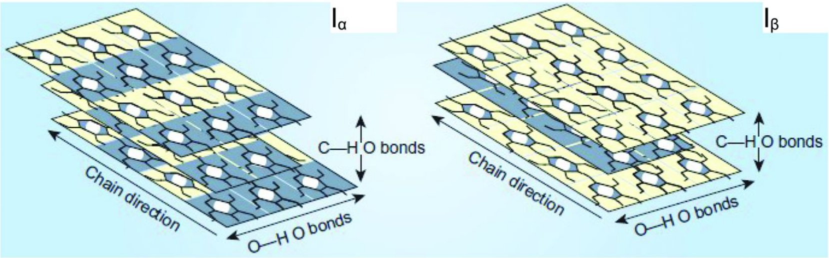

Figure 3. The stacking pattern of linear cellulose chains. (Reproduced with kind permission (19) and Copyright of Nature)

As shown in Figure 3, all cellulose chains lie parallel bonded through O-H…O edge to edge. The sheets of these chains are stacked on top of one another with a stagger that differs between I_α and I_β forms. These sheets are bonded through multiplicity of weak C–H•••O hydrogen bonds. Though being hydrophilic, cellulose has strong resistance to acid-hydrolysis which could be partially due to the presence of multiple H-bonds formed between adjacent chains (*20*). The degree of polymerization of cellulose ranges between 10,000 and 15,000. Cellulose from different lignocellulosic origin has the same chemical composition however deferring in the degree of polymerization, crystallinity and crystalline forms, hence, different mechanical properties. Cellulose provides reinforcement to the structure of lignocellulose, generally, the higher the cellulose content the greater of the mechanical strength of lignocellulose. Cellulose contains hydroxyl functional groups and it has an equilibrium moisture content of 8-12.6%.

Cellulose Nanocrystallites

Cellulose nanocrystallites (CNs) can be isolated from bacteria (bacterial cellulose), lignocellulose and tunicates (tunicates cellulose). The CNs, also known as "whiskers", has characteristic thin and long crystalline morphology. Bacterial cellulose is synthesized by *Acetobacter xylimum* on a nutrient media containing glucose. It is embedded in a matrix of protein, free of lignin and hemicellulose. The network structure of bacterial cellulose has a bulk density of about 1.6 g/cm^3 (*21–26*). It can be used for making high performance composites, special technical paper, diaphragms for audio speakers or headsets, and even as wound dressings, artificial skins and other biomedical devices due to biocompatibility (*27–35*). The CNs isolated from lignocellulose is about 5-50 nm in width and about 100-500 nm in length depending on lignocellulosic origins (*36–41*). Tunicates cellulose is about 10-20 nm in width and 500-2000 nm in length (*42*). Bacterial cellulose is about 10-50 nm in width and 100-1000 nm in length (*43*). TEM images of selected CNs are shown in Figure 4. It is generally believed that CNs isolated from lignocellulose are flat with uniplanar-axial orientation. Bacterial cellulose (*43*) and tunicates cellulose (*44*) have twisted ribbon-like shapes with half-helical pitches of about 600-800 nm and about 1.2-1.6 μm, respectively. The CNs have modulus around 100-140 GPa (*45–47*). Aspect ratio, defined as the length-to-width ratio, is about 10-30 for cotton and about 50-100 for tunicate. The large aspect ratio and nanoscale dimensions of CNs may explain their unique mechanical, optical, electrical and chemical properties that are not found in macroscopic cellulose.

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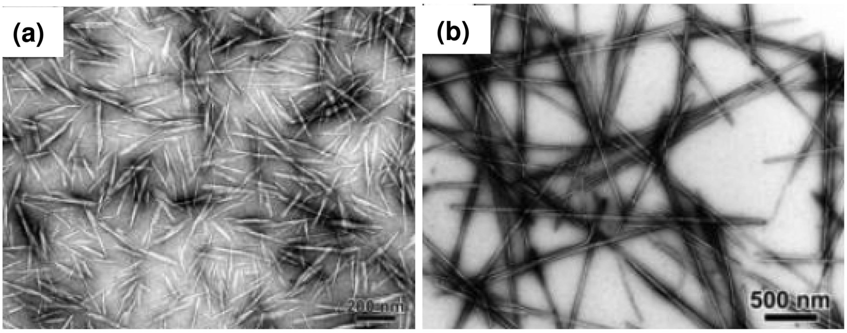

Figure 4. Network structure of CNs isolated from (a) Ramie lignocellulose. (Reproduced with kind permission of (37). Copyright 2008 The Royal Society of Chemistry) (b) Tunicates (Reproduced with kind permission of (44). Copyright 2008 American Chemical Society).

Regenerated Cellulose

Regenerated cellulose, also known as Rayon, has the same chemical composition as natural cellulose. It usually has high luster quality giving it bright sheen. It can be yarned and threaded, making it a good raw material for both woven and non-woven industries. Regenerated cellulose is biodegradable. It has high moisture regain, dimensional stable to heat, dyes easily, retains strength when wetted and have predicable mechanical and morphological properties which are superior comparing to plant cellulose. Regenerated cellulose has three major brands including Lyocell/TENCEL®, Modal® and Viscose®. Lyocell has comparable mechanical properties to flax, jute, sisal and hemp (*48*, *49*).

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Isolation of Biofibers

Lignocellulose can be broken down into fiber bundles and single fibers by grinding and refining. In the grinding process, lignocellulose is mechanically broken down into fibers using rotating plates in a wet environment at a fiber yield close to 100%. If the refining is done at high temperatures, fibers tend to slip apart due to the softening of lignin matrix between the fibers rendering them a lignin-rich surface. If the refining is done at low temperatures, fibers tend to break apart rendering them a carbohydrates-rich surface. Chemical composition of mechanically processed fibers remains close to that of the original lignocellulose, namely cellulose, hemicelluloses and lignin. Refined lignocellulosic fiber is more hydrophilic due to fibrillation.

Cellulose is isolated from lignocellulose mainly through chemical processing (soda, sulphite or sulphate), known as chemical pulping. Chemical processing dissolves lignin, hemicelluloses and other cell wall components, releasing cellulose of varying purity depending on the type and conditions of chemical processing. Fiber yield of the chemical processing is generally around 40-50% due to the loss of lignin, hemicelluloses, pectin, wax and other small cell wall components.

Method most adopted for the isolation of ***CNs*** from natural resources is by acid hydrolysis. Amorphous domains of lignocellulose are preferentially hydrolyzed whereas crystalline cellulose having higher resistance to acid attack remains intact. The CNs may exhibit different morphology, surface charges and varying amount of amorphous cellulose residues depending on the hydrolysis conditions (time, temperature, choice of acids). Sulfuric and hydrochloric acids are commonly used to isolate CNs (*50*, *51*). If the CNs is isolated using hydrochloric acid, their aqueous suspensions tend to flocculate resulting in poor dispersion. On the other hand, when sulfuric acid is used as a hydrolyzing agent, it reacts with the surface hydroxyl groups of cellulose giving rise to charged sulfate esters that facilitate the dispersion of the CNs in aqueous suspension. Combining high shearing mechanical refinement and mild enzymatic hydrolysis lead to microfibrillated cellulose (MFC). Generally, MFC consists of nanofibril aggregates of about 10-30 nm in width (*52*).

Regenerated cellulose is chemically regenerated from natural cellulose by reorienting cellulosic chains along the fiber axis and allowing the cellulosic

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

chains to cross-bond as they become parallel to each other. The three brands of regenerated cellulose (Lyocell/TENCEL®, Modal®, Viscose®) represent three different manufacturing processes with a common root going back to France in the 1900s to produce a textile known as artificial silk (*eq.*1).

$$(C_6H_{10}O_5)_n + nNaOH \rightarrow (C_6H_9O_4ONa)_n + nH_2O$$
$$(C_6H_9O_4ONa)_n + nCS_2 \rightarrow (C_6H_9O_4O\text{-}SC\text{-}SNa)_n$$
$$(C_6H_9O_4O\text{-}SC\text{-}SNa)_n + nH_2O \rightarrow (C_6H_{10}O_5)_n + nCS_2 + nNaOH$$
$$(C_6H_9O_4O\text{-}SC\text{-}SNa)_n + \tfrac{1}{2}nH_2SO_4 \rightarrow (C_6H_{10}O_5)_n + nCS_2 + \tfrac{1}{2}nNa_2SO_4 \qquad (eq.1)$$

Biofibers in Application

Biofiber Boards

As the availability of large diameter trees decreased and price escalated, the wood industry looked to replace large timber and solid lumber with reconstituted wood boards made using smaller diameter trees, saw dust, recycled and non-wood biofiber feedstock. The reconstituted boards started with very thick laminates for glue laminated beams, to thin veneers for plywood, to strands for strandboard, to flakes for flakeboard, to particles for particleboard, and finally to biofibers for fiberboard. As the size of the constituent element gets smaller, it is possible to either remove defects (knots, cracks, checks) or redistribute them to reduce their effect on the properties of boards. And more significantly, the reconstituted boards become more like a true material, *i.e.* consistent, uniform, continuous, predictable and reproducible.

The advantages of biofiber boards are evident (i) reduce reliance on wood, (ii) leverage on the diversified non-wood biofibers, (iii) remove defects, (iv) engineer biofiber boards to deliver desirable strength and functionality (v) mold three dimensional biofiber profiles.

Biofiber boards are assembled materials of lignocellulose and adhesive through cross-linking. A biofiber board can be formed using a wet forming or a dry forming process. In a wet forming process, lignocellulose is distributed via water into a mat and then pressed into a flat board. In many cases, the lignin of lignocellulose serves as adhesive, hence, no adhesive is necessary. In the dry process, mechanically refined lignocellulose goes through a dryer and blow line where adhesive is applied, and formed into a web which is then pressed into a flat board.

Biofiber boards are classified based on the density of boards. Low density board (LDF density: 0.15-0.45 g/cm^3) is used for sound insulation in walls and ceilings, medium density board (MDF density: 0.6-0.8 g/cm^3) is used as furniture cores and wall structures, and high density board (HDF density: 0.85-1.2 g/cm^3) is used as structural overlays.

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Adhesion

Synthetic adhesives have been largely used in the manufacturing of biofiber boards to render them with good mechanical properties. Common adhesives include melamine formaldehyde (MF), urea formaldehyde (UF), melamin-urea-formaldehyde (MUF), phenol formaldehyde (PF) and methylene diphenyl diisocyanate (MDI). Typical adhesives account for about 10-15% of the board mass. In fact, these adhesives are formulated based on the chemistry and physical characteristics of wood lignocellulose, hence, direct application of these adhesives for non-wood lignocellulose boards does not usually produce satisfactory result (*53–55*). For example, straw lignocellulose is covered with a waxy cuticle that inhibits its adhesion with water-based adhesives, and also interferes the hardening of aminoplastic resins. Emission of free formaldehyde during board production and service life of the biofiber boards containing formaldehyde adhesives (UF and MUF) is of health concern (carcinogenic to humans) among consumers and manufacturers (*56*, *57*). A further issue is that these adhesives are thermoset; hence, cured thermoset adhesives limit the reuse options for discarded boards and production rejects (*57*, *58*).

One technical solution to tackle one of above issues is to use MDI thermoset resin for wheat straw boards (*59–61*). MDI has advantages over formaldehyde resins for its low resin dosage and good bonding strength, however, the large scale adoption is hampered by its high cost and tendency to adhere to metal surfaces. Researchers at the Washington State University have developed a low cost adhesive formulation based on formaldehyde resins and wax, and the wheat straw boards produced using this proprietary adhesive have passed stringent requirements of American National Standards Institute (ANSI) (*62*). Researchers at Mild Sweden University and Metso Corporation have jointly developed a composition/process combination for making MDF and HDF from rice straw using MDI adhesive. Their study reveals that rice straw boards produced using their invention have excellent flexural properties, internal bond strength and thickness swelling, meeting the requirements of MDF for interior applications (*63*).

Hoareau and coworkers developed a lignin-PF adhesive formulation containing 40% to 100% wt lignin extracted from sugarcane bagasse (bagasse). It is revealed that the lignin-PF adhesive is suitable for making bagasse boards. Their study also reveals that chemical modification of bagasse renders the bagasse boards improved resistance to white rot fungi attack (*64*).

The quest for a formaldehyde-free biofiber board has led to invention of a number of sustainable adhesive formulations based on starch, soy protein and lignin (*65–69*). Laboratory test results of using these adhesives for biofiber board production are encouraging.

Auto-Adhesion

Concurrent application of heat and pressure on lignocellulosic fiber mat in a closed press for a few minutes leads to formation of dimensional stable

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and moisture resistant biofiber boards, known as auto-adhesion (*70–73*). Auto-adhesion of lignocellulose is the result of chemical reactions involving lignin and hemicelluloses where hemicelluloses break down to cross linkers, and lignin undergo acid-catalyzed condensation reaction. The binding strength rendered by auto-adhesion is often insufficient to totally displace synthetic adhesives.

One promising approach to enhance the auto-adhesion strength is through radical condensation of lignin using phenol-oxidizing enzymes (laccase and peroxidases) (*74*). The mechanism of the enzyme-facilitated auto-adhesion involves the generation of phenoxyl radicals located on fiber surface (*75–85*). Studies reveal that laccase-treated lignocellulose has different chemical composition and exhibits remarkably improved hydrophobicity. The biofiber boards made of laccase-treated lignocellulose have greater wet strength as compared to untreated biofiber boards. This could be due to the formation of lignin macromolecules through radical condensation. Nevertheless, contributions to the adhesion by condensation of hemicelluloses degradation products, molecular entanglement and even hydrogen bonding should not be ruled out.

The properties of MDF prepared in laboratory-scale and pilot-scale from laccase-treated lignocellulose of wood and non-wood show a noticeable improvement (*75*, *77*, *82*). Proprietary technologies relating to binderless fiberboards manufactured from laccase-treated lignocellulose are disclosed in patents (*83*, *84*).

Technologies have been patented on the use of laccase-treated technical lignin such as kraft lignin or lignosulfonate as adhesives to partially replace synthetic resins (*85–87*).

Biofiber Modification

Dimensional instability, flammability, biodegradability, and degradation caused by acids, bases and ultraviolet radiation of lignocellulose can be prevented or, at least, slowed down if the cell wall chemistry is altered (*88–90*). Acetylation of lignocellulose has proven to be viable and effective in extending the service life of lignocellulose boards (*91–95*). The one in commercial production is based on the reaction of acetic anhydride with surface hydroxyl groups of lignocellulose (*96*). Rowell and coworkers reveal that acetylation of pinewood lignocellulose reduces the equilibrium moisture content from 18.9% of untreated MDF to 3.1% of treated MDF. Thickness swelling of MDF made of acetylated pinewood lignocellulose is less than 5% after 5 days comparing to 36.2% of MDF made of untreated pinewood lignocellulose. The wet strength and wet stiffness of MDF made of acetylated pinewood lignocellulose are higher by 62% and 140% respectively than MDF made of untreated pinewood lignocellulose.

Very low thickness swelling of acetylated straw MDF (< 2%) has been reported by Gomez-Bueso (*97*). Acetylation of lignocellulose improves its hydrophobicity and dimensional stability at the expense of mechanical properties. This is due to the fact that acetylated lignocellulose has reduced wettability with water-based adhesives. The wettability of acetylated lignocellulose can be altered with the aid of wetting agents. It is reported that treating acetylated lignocellulose using gas phase ozonation improves its wettability with water-based adhesives. Using this method, MDF produced in laboratory scale exhibits significantly improved dimensional stability at no loss of mechanical strength (*98*). Östenson and coworkers study the surface of acetylated lignocellulose during gas phase ozonation and reveal that low molecular weight fragments containing carbonyl and carboxyl groups are formed as a result of degradation of acetylated lignocellulose (*99*).

Molded Biofiber

Molded biofiber is conventionally manufactured from cellulose using a paper-making process (*100*). Cellulose can be virgin, recycled, bleached and unbleached. Barrier properties of a molded biofiber is commonly achieved using internal furnishing additives such as Alkyl Ketene Dimer (AKD) and rosin-based sizing to render water barrier, polyamide-epichlorohydrin to render wet strength and fluoro-polymers to render grease barrier. A molded biofiber made of virgin cellulose is about 0.6-1.5 mm in thickness and light-weight with moderate structural rigidity suitable for food packaging; a molded biofiber made of recycled cellulose usually has low density with moderate cushioning properties suitable for protective applications.

Structural molded biofiber can be made using a similar process. Setterholm (*101*) develops a method for making a three-dimensional waffle-like structure using a hard flexible rubber forming head. The product, known as “Spaceboard” shown in Figure 5, can be used for structural applications. Further advancement has been made by Hunt (*102*) and Scott (*103*).

A molded biofiber contains hydrogen-bonded network of cellulose. Upon exposure to high moisture environment, conventional molded biofiber deforms and shows signs of wetting due to formation of competing hydrogen bonds between cellulose and water which weakens the inter-cellulose hydrogen bonds.

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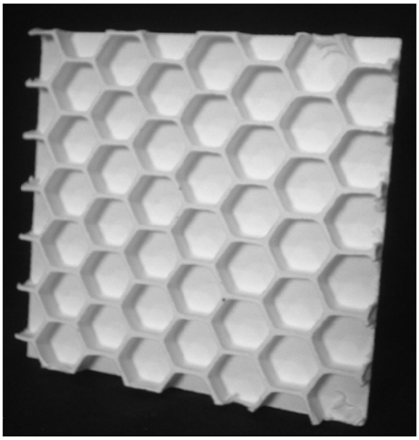

Figure 5. Molded biofiber “spaceboard” for structural application.

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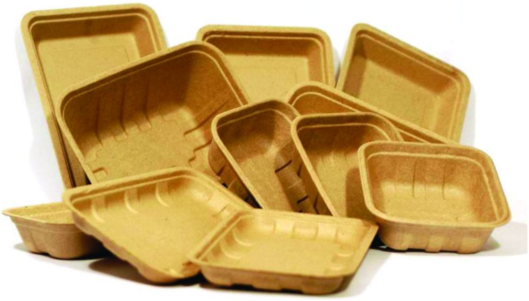

Figure 6. Molded biofiber made of oil palm lignocellulose.

Lignocellulose has been regarded a less desirable feedstock for molded biofiber due to its high lignin content and lack of surface hydroxyl groups. Enabling auto-adhesion among lignocellulose is a promising area worthy of exploration (*75–79*). Challenges remain to be a process that allows thermoplasticization to happen in a reasonable cycle time, and processing temperatures and pressures (*104*). Commercial realization of above principal has been reported by Grenidea Technologies p/l. Molded biofiber made of oil palm lignocellulose is manufactured using paper-making process where the lignocellulose is mechanically isolated from oil palm EFB. A proprietary starch binder is added to the lignocellulose formulation to render the molded biofiber with strength and liquid holdout properties (*105*). The molded biofiber prepared using this method meets the requirement of international standards (DIN EN 13432/OK Compost/BPI) for 100% compostable via biodegradation (Figure 6).

Compounding lignocellulose with polymeric resins via extrusion, injection molding and non-woven process enables the assembly of a wide range of performance biocomposites. Applying this concept to molded biofiber process leads to a new class of molded biofiber made of lignocellulose and cellulose of various origins, and thermoset adhesives or heat fusible binders such as polyethylene (PE), polypropylene (PP) grafted with maleic anhydride (MA) (*106–108*).

Molded lignocellulose containing heat fusible binders exhibit significantly improved mechanical properties. Choice of thermoplastic binders decides the physical properties such as tensile strength, stiffness, breaking length, RCT and porosity of the molded lignocellulose. Heating lignocellulosic composition above the melting temperature of heat-fusible binder bonds lignocellulosic compositions together. Thermoplastic binders in either pulp or fiber form improve the wet-tensile and wet-stiffness of the molded lignocellulose as shown in Table IV. It is interesting to note that binder pulp enhances the surface smoothness and bending stiffness while binder fiber improves the tensile strength and breaking length of molded lignocellulose. Regenerated cellulose has a positive effect on the porosity of molded lignocellulose. Similar enhancement on mechanical properties can be achieved with the use of thermoset binders except for surface porosity, as shown in Table V.

Table IV. Mechanical properties of molded oil palm lignocellulose containing thermoplastic binders

	Control	*PE pulp*	*PE fiber*	*PP fiber*	*Regenerated cellulose fiber*
Dry tensile index, Nm/g	9.78	14.46	16.33	22.36	13.33
Wet tensile index, Nm/g	2.23	4.71	5.07	5.28	3.71
Bending stiffness, mN	108	150	124	126	122
Breaking length, m	980	1475	1665	2259	1354
RCT, N	120	533	562	617	293
Porosity, ml/min	1230	505	616	566	1600

Table V. Mechanical properties of molded oil palm lignocellulose containing thermoset binders

	Control	*Modified starch*	*Styrene-butadiene copolymer latex*	*Self-crosslinked acrylic latex*
Dry tensile index, Nm/g	9.78	12.21	15.56	13.41
Wet tensile index, Nm/g	2.23	2.58	3.54	2.12
Bending stiffness, mN	108	136	81	92
Breaking length, m	980	1245	1587	1367
RCT, N	120	355	466	366
Porosity, ml/min	1230	1150	1064	2615

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Inspired by prior work on auto-adhesion of lignocellulose (*75–79*), researchers at Grenidea Technologies p/l have developed a method for improving the wet-strength of molded lignocellulose by pretreating oil palm lignocellulose using laccase (*109*). Molded lignocellulose prepared this way is suitable for packing the most aggressive fresh produce, mushroom.

Conventional molded biofiber process is a wet process which limits materials formulation and is also energy consuming. Frederiksen and coworkers modify conventional molded biofiber process by replacing wet forming with air-laid forming to form biofiber non-woven profiles, followed by thermal molding to give rise to three-dimensional molded biofiber (*110*). This invention enables the assembly of multi-layer molded biofiber that are unprecedented for conventional molded biofiber.

The above work is commendable as it extends conventional molded pulp to biofiber composite formulation by taping on a wide range of lignocellulose and polymeric binders using air-laid forming and thermal molding. It is acknowledged that compounding technology for fiber-reinforced composites has been optimized to a reactive extrusion process (*8*, *111*, *112*). Much work needs to be done to evaluate whether the paper-making process can be modified to fit to lignocellulose/ polymer compositions, or if a different surface modification for lignocellulose and polymers needs to be developed to meet the requirement of conventional paper-making process.

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Biofiber Nonwoven

A biofiber nonwoven is formed from biofiber and polymers through processes including carding, either wet-laid or air-laid, and needle punching. A carded mat consists of mechanically oriented and interlocked fibers which are bonded through mechanical entanglement and chemical bonding. A wet-laid mat is made using a modified paper making process to take advantage of its production speeds. Wet-laid mats are generally flexible, bulky, drapeable, porous and have some degree of surface roughness (*113*). Approximate 5-10% of nonwovens are made using wet-laid forming technology. Among many applications, wet-laid nonwovens can be used as filtration medium such as micro-glass paper, tea bags, coffee filters and battery separators (*114*).

Modern air-laid technology is inspired by the early air-laid machines of Rando and Danweb, and has now evolved to be a highly versatile and energy-saving technology for the production of flexible and rigid nonwoven (*115–118*). An air-laid mat is formed by laying layers of biofiber and low melt thermoplastic fibers, and followed by pressing the layers of fiber mix through heated calendar roll. Air-laid process yields paper-like mats with good tear resistance and wet tensile strength. They are suitable for many disposable absorbent applications such as diapers and soaker pads (*119*). The density of the biofiber nonwoven can be controlled by varying biofiber compositions and processing conditions.

Wood lignocellulose has high water absorbency, bulk and low cost hence it is a preferred feedstock for making biofiber nonwoven. Non-wood lignocellulose is emerging due to its unique properties that can be used for specialized application, however, quality variation, unstable pricing and supply inconsistency hamper their widespread adoption. Cotton, regenerated cellulose and cellulose acetate (a cellulose ester of cellulose and acetic acid) are absorbents and can be used as carriers for microbial agents. Among polymers, PP has been widely used due to its low cost, easily processable and voluminous. Polyethylene terephthalate (PET) is used when mechanical property is of primary importance. Nylon is used for its resilience. Bicomponent fibers with different polymers in the core and sheath are often used in thermally bonded nonwovens. The choice of fibers for nonwovens depends on the product requirement and the fabrication process.

A biofiber nonwoven is bonded through mechanical interlocking and chemical bonding where latex, heat fusible binder fibers or a combination of both are commonly used. Water-based latex, such as polyacrylate, styrene-butadiene polymer, ethylene-vinyl acetates, vinyl chlorides, are common latex binders for wet-laid nonwoven. Latex bonding agents could account for 30% or more of a nonwoven composition (*114*). Low melt polymeric fibers are often blended into biofiber composition as structural elements as well as binding agents to provide additional bonding and to render the biofiber nonwoven with required stiffness. Typical polymeric fibers are vinyon, PP, cellulose acetate, and special low melt polyester or polyamide copolymers.

The biofiber nonwoven can be used as produced such as biofiber geotextiles for erosion control and mulching mats or compression molded to produce molded composites (*120*, *121*), as shown in Figure 7. Molding biofiber nonwoven to produce three-dimensional profiles has been well received, especially in the packaging and automobile industries. Uniform thickness can be achieved and almost any shape is possible. Chemical compatibility, interfacial bonding and susceptibility to moisture of biofibers remain to be challenging (*122*, *123*).

Biofiber nonwoven has found applications as filtration materials for water purification. Laszlo and Dintzis (*124*) have shown that lignocellulose has ion-exchange and adsorption capacity, due to its composition and hierarchical structure. The polymers including extractives, cellulose, hemicelluloses, pectin, lignin and protein are adsorbents for a wide range of solutes, particularly divalent metal cations. Lignocellulose contains polyphenolic compounds, such as tannin and lignin, which are believed to be the active sites for heavy metal cations (*125–134*). Metal ions compete with hydrogen ions for active sorption sites on lignin. Previous studies also reveal that metal sorption onto lignin is dependent on both sorption time and metal concentration. Basso and coworkers (*135*) study the correlation between lignin content of wood lignocellulose and their ability to remove heavy metals from aqueous solutions. The efficiency of removing Cd(II) and Ni(II) from aqueous solutions is measured and a direct correlation between heavy metal sorption and lignin content is observed. Reddad and coworkers (*136*) show that the anionic phenolic sites in lignin have high affinity for heavy metals.

Biofiber nonwoven filters are able to remove suspended solids from air and water (*137*). Shin and coworkers (*138*) have shown that phosphorus can be removed from water using a nonwoven filter made of juniper lignocellulose. Figure 8 shows a filter system being installed to remove heavy metals from an acid mine site in Ohio.

Kapok is a low density, high porosity and hydrophobic–oleophilic plant fiber with great surface area. Due to its inherent characteristics, kapok enables water to be filtered through while retaining oils. Huang and coworkers study the filtration efficiency of kapok for the removal of diesel and hydraulic oil from oily water samples, and report filtration efficiency of 100% and more than 99.4% for diesel and hydraulic oil respectively (*139*).

Nonwoven filters made of citric acid treated flax at no less than 75% flax loading can be used to remove copper ion. However, the diminution in fabric strength after treatment has yet reached satisfactory for commercial adoptions (*140*).

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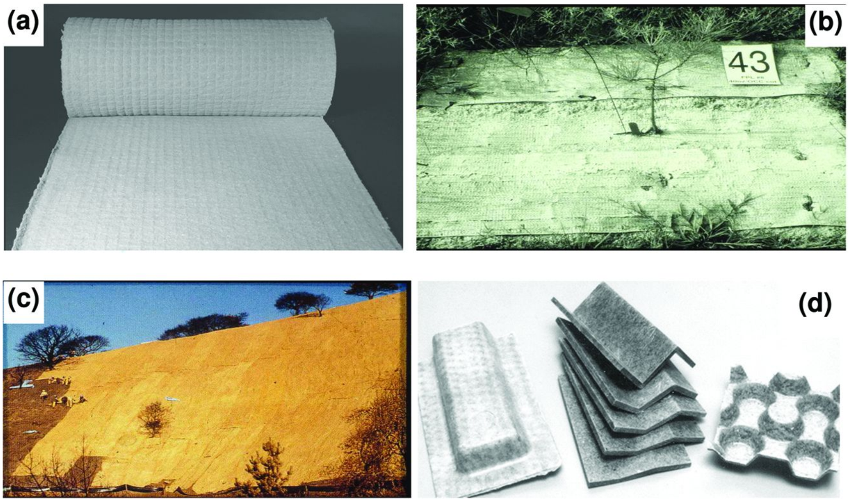

Figure 7. Biofiber nonwoven and their products (a) Danweb fiber web (b) Mulching mat (c) Geotextile (d) Molded biofiber.

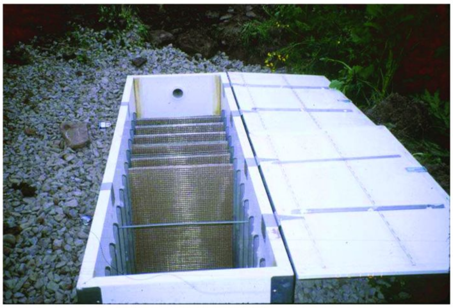

Figure 8. A nonwoven filtration system made of wood lignocellulose to remove heavy metals from contaminated water.

Lignocellulose has porous structure and high surface volume. Water is able to penetrate though the amorphous region of cellulose, hemicelluloses and lignin. Lignocellulose nonwoven could potentially become an economic and sustainable solution for water purification especially in rural areas where a high-technology water purification system is not affordable, however much work needs to be done before it can become a viable and competitive solution (*141–146*).

Biofiber-Reinforced Composites

Biofiber replacing glass and carbon fiber as reinforcement fillers for polymeric matrix affords a new class of materials (*147*). Biofiber is favored over synthetic and mineral fibers due to its low density, high specific strength, non-abrasiveness, good acoustic and thermal insulating properties in addition to its environmental benefits and abundant availability. Recent advancements in biofiber-reinforced composites (BRC) are reviewed (*5*, *148–151*). A BRC consists of three phases (i) continuous matrix phase (ii) discontinuous reinforcement phase (iii) interfacial phase. The advancement of BRC is performance-driven. Principle chemistry issues include interfacial bonding and functional design (*152*).

Selection of Reinforcement Biofiber

Criteria for choosing a biofiber as reinforcement filler include compatibility (chemical, physical, mechanical) between biofiber and matrix, supply sustainability, price, processability, processing cost and functional requirement, *etc*. A logical approach is to compare the chemistry and key mechanical properties of biofiber with proven reinforcement fillers such as glass and carbon. As shown in Figure 9, biofibers such as hemp, flax, cotton can potentially replace glass, steel for applications.

Biofibers are hydrophilic and organic polymers are often hydrophobic. Commodity thermoplastics most studied and some found in commercial BRC include PP, PE, low density polyethylene (LDPE), high density polyethylene (HDPE), polystyrene, polyvinylchloride among which PP is more commonly used. These thermoplastics have softening temperatures below the degradation temperature of lignocellulose (200-210°C). PP has softening temperature about 190 to 200 °C which limits its applications in BRC design because when improperly processed it may harm biofibers. PE is a popular choice of polymeric component for BRC due to its processing temperature which is grossly below the degradation temperature of lignocellulose; besides PE comprises more than 70% of total plastic waste which, when efficiently recycled, could turn huge amount of solid waste to profitable commodity products.

Sgriccia and coworkers study the reinforcement effect of kenaf and hemp on epoxy, and compare the results with that of glass fiber. It is found that silane-treated kenaf out-performs glass fiber as reinforcement filler for epoxy as evidenced by significantly improved flexural modulus. Kenaf appears to be a better reinforcement than hemp for epoxy matrix under the same treatment conditions (*154*, *155*).

The reinforcement behavior of ramie on poly lactic acid (PLA) has been studied in an attempt to design fire retardant biocomposites by Li and coworkers. In their study, ramie and PLA are respectively treated with a flame retardant, ammonia phosphate (APP), prior to compounding, and a silane is used to enhance the ramie/PLA interfacial adhesion. It is found that the ramie/PLA composite has optimal mechanical/flame retardant performance at (30%ramie+5.3%APP):(59.5%PLA+5.2%APP). It is reported that APP-treated ramie enhances its reinforcement effect on PLA and APP-treated PLA deteriorates the mechanical properties of ramie/PLA biocomposites (*156*).

Lignocellulose isolated from tropical plants such as oil palm EFB, banana stalk, PALF and coir is inexpensive and available all year around, however, insufficiently understood. Limited studies suggest that treating banana

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lignocellulose using alkylization, silane, cyanoethylation, heat and latex improve its reinforcement effect on PF to varying degree. (*157–159*).

Abaca, also known as Manila hemp, is a waste product of banana cultivation. Abaca lignocellulose is rotting-resistant and has high tensile strength. Its specific flexural strength is close to that of glass fiber (*160*). Recent surge in designing abaca-reinforced composites is inspired by DaimlerChrysler's commercial release of abaca/PP floor mat for its passenger cars. Abaca lignocellulose is the first non-wood lignocellulose meeting the stringent quality requirements for components used as the exterior parts of road vehicles (*161*).

Lignocellulose isolated from PALF show positive reinforcement effect on PLA. Huda and coworkers prepare PALF/PLA laminate composites using film stacking method. It is found that PALF improves the stiffness and heat defection temperature (HDT) of PLA. Treating PALF with silane coupling agent and alkali enhances its adhesion with PLA, and significantly improves the mechanical properties of PALF/PLA composites. Siregar and coworkers study the reinforcement behavior of PALF over a range of polymeric matrices and report that the mechanical properties of alkali-treated PALF/polystyrene is optimized when treated using 4% NaOH solution (*162*, *163*).

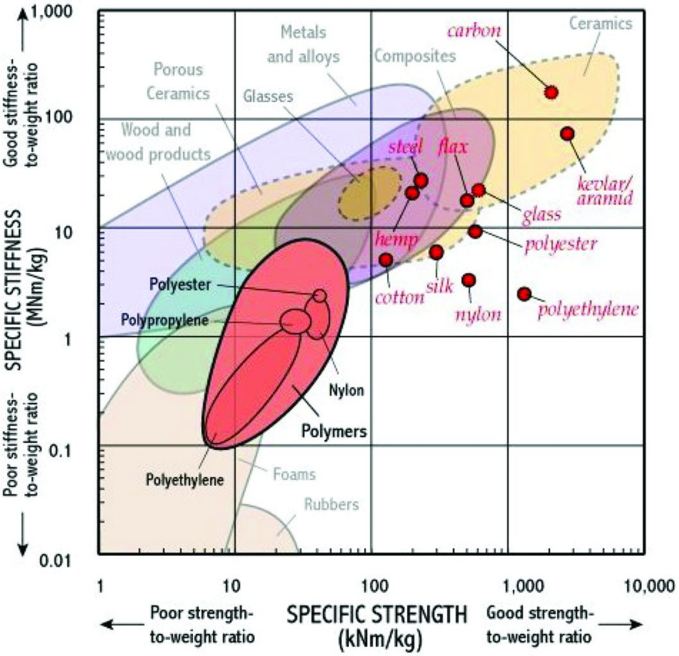

Figure 9. Comparing biofibers with inorganic and organic fibers. (Reproduced based on public source (153))

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Coir dust is a waste generated from production of coir lignocellulose, and it contains a mixture of short lignocellulose fiber and powder. In a specific research, coir dust is used to reinforce polyhydroxybutyrate (PHB). It is found that coir/PHB composites containing 10 wt% coir dust improves the tensile strength by 35% and elongation at break by 25% compared to neat PHB. Higher loading of coir dust results in poor dust dispersion and agglomeration. The study reveals that neat coir dust does not adhere well with PHB, suggesting surface treatment of coir dust may be needed to enhance the interfacial adhesion between coir dust and polymeric matrix (*164*).

Oil palm EFB is a process waste of palm oil production. It is a valuable lignocellulose feedstock that has yet to be sufficiently understood. Malaysia alone produces approximately 2.9 million tons of oil palm EFB lignocellulose annually (*165*). Early studies reveal that oil palm EFB exhibits reinforcement effect on rubber, PP and HDPE, *etc.* The alkylization of oil palm EFB and the use of compatibilizers enhance the adhesion between oil palm EFB and polymeric matrices, hence improving the mechanical properties of oil palm EFB-reinforced composites (*165*, *166*).

Biofiber Treatment

Inherently, lignocellulose and cellulose have polar and hydrophilic surface, non-uniform geometry, and varying mechanical properties. They are susceptible to biological attack and thermal degradation at temperatures above 200 °C. This poses technical challenges to their dispersion in polymeric matrix and hinders the development of satisfactory interfacial bonding with polymeric matrix. This situation can be partially improved through surface treatment as reviewed (*10*, *167–171*). Broadly biofiber treatment can be classified into (i) physical treatment, (ii) dissolution, (iii) chemical modification. Often these methods are combined in one way or another with an ultimate goal of improving compatibility and wettability between biofibers and polymeric matrices.

a. Physical Treatment

Commonly used physical treatment methods include (i) mechanical diminution, (ii) heat treatment, (iii) steam explosion treatment.

(i) Mechanical refinement. It is achieved by passing 4-6% lignocellulosic slurry between rotating and stationary discs or cones. Mechanical refinement peels off the cell wall of lignocellulose and progressively unravels sub-layers. It increases the surface area and surface hydroxyl groups, and improves the flexibility and collapsibility of lignocellulose (*172*). Reduction in the dimension of lignocellulose may cause a change in the degree of polymerization (*173*). Disadvantage of the mechanical refinement includes high energy consumption, damaged fiber morphology and non-uniform fiber dimension.

Mutje and coworkers study the effect of mechanical refinement of hemp lignocellulose on the mechanical properties of injection-molded hemp/PP

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composites. Their study reveals that refining hemp significantly improves the mechanical properties of hemp/PP composites, typically the specific mechanical properties of the hemp/PP composites may achieve about 80% 80% that of glass fiber/PP (*174*).

(ii) Heat treatment. Heating lignocellulose between 180-250 °C causes a series of chemical reactions to take place as reviewed (*73*, *175–177*). This includes depolymerization and recondensation of lignin, hydrolysis of hemicelluloses, removal of extractives and formation of new extractives due to the degradation of cell wall components. The hydrolysis of hemicelluloses generates acids which further catalyze the hydrolysis of hemicelluloses. When heated above 250 °C, lignocellulose undergoes carbonization forming CO_2 and other pyrolysis products.

(iii) Steam-explosion treatment. Steam explosion employs quick depressurization and cooling down of pre-steamed lignocellulose to cause encapsulated water in the lignocellulose to explode. Steam explosion depolymerizes lignin; cleavages hemicelluloses-lignin bonds and hydrolyzes hemicelluloses (*170*, *178*). It is worth mentioning that steam explosion causes re-condensation of lignin, as a result, increases surface hydrophobicity and improves lignocellulose/polymer adhesion. Steam explosion could partially remove amorphous cell wall components such as pectin, hemicelluloses and lignin, reduce refinement energy and increase the crystallinity of cellulose.

In an interesting research, Renneckar and coworkers modify wood lignocellulose by co-steam exploding wood lignocellulose/polyolefines (PE, PP). It is reported that co-steam explosion of wood lignocellulose/polyolefins reduces water sorption rate where the degree of reduction is a function of polyolefin loading and polyolefin type. Reports show that more significant reduction in water sorption rate is obtained in co-steam explosion of wood lignocellulose/PP than that of wood lignocellulose/PE. Reduction in water sorption rate is a reflection of the change in diffusion constant, suggesting a possible modification of the cell wall structure of lignocellulose (*179*).

Steam treatment of alkali-treated lignocellulose may further improve the tensile strength of the lignocellulose. In one study, Saha and coworkers report that dipping jute lignocellulose in 0.5% alkali solution for 30 minutes followed by 30-minute alkali-steam treatment increases the tensile strength of the jute lignocellulose by 65%. This increase may be due to the removal of amorphous cell wall components, improvement in crystallinity and better fiber separation (*180*). Similar improvement is observed for banana lignocellulose in a process involving alkaline pulping and steam explosion (*181*).

b. Dissolution

It refers to processes used to remove extractives and amorphous cell wall components from lignocellulose. Common methods include (i) alkylization, (ii) enzymatic treatment.

(i) Alkylization. Also known as alkali treatment or mercerization, alkylization removes amorphous cell wall components (wax cuticle layer, part of lignin, pectin, hemicelluloses, amorphous cellulose), reduces the size of fiber bundles,

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roughens fiber surface and improves the crystallinity of cellulose. It reduces the diameter of lignocellulose, exposes hydroxyl groups, causes a denser and more thermodynamically stable cellulose structure to form and often improves the lignocellulose-polymer adhesion (*180*, *182–186*).

The alkylization of non-wood lignocellulose of hemp, sisal, jute, kenaf, oil palm EFB and coir show consistent improvement in the tensile and flexural strength, and tear resistance of BRC (*169*, *187–190*). Hai and coworkers report that the tensile strength and modulus of alkali-treated jute lignocellulose/PP composites can be improved up to 40% and 9%, respectively. Alkali-treated coir lignocellulose/PP composites exhibit the best improvement in the tensile strength and modulus by 62% and 17%, respectively. Treating lignocellulose using 2% NaOH solution enhances the elongation at break by 8% for jute lignocellulose/PP and by 13.5% for coir lignocellulose/PP composites. The moisture absorption for the jute lignocellulose/PP and coir lignocellulose/PP composites reduces by 50% and 60% comparing to their untreated/PP composites, respectively (*188*).

(ii) Enzymatic treatment. Studies show that cellulase, hemicellulase and pectinase facilitate the separation of non-wood lignocellulose of flax, kenaf and coconut hull (*191*). Cellulase promotes the liberation of long and rod-like lignocellulose (*192*). Pectinase (polygalacturonase) plays a key role in the separation of lignocellulose (*193*, *194*). Pectinase can effectively degrade pectin, hence weakening the adhesion among fibers and fiber bundles in the middle lamella of cell wall structure. It is evident that enzymatic treatment enable the removal of extractives and amorphous cell wall components from lignocellulose (*195–197*).

Gulati and coworkers treat hemp lignocellulose using a fungus obtained from elm tree infected with Dutch elm disease and compound the treated hemp lignocellulose into an unsaturated polyester matrix. It is observed that this treatment improves hemp's resistance to moisture and dispersion in polymeric matrix. The fungus-treated hemp lignocellulose/polyester composites show improvement in flexural strength and flexural modulus comparing to un-treated hemp lignocellulose/polyester composites (*195*).

An all-cellulose composite is assembled from cellulose acetate butyrate (CAB) and hemp. To improve interfacial adhesion, CAB is plasticized with tributyl citrate (TBC) at loading of 10-30 vol%, and hemp is pre-treated using pectinase. It is found that pectinase-treated hemp/CAB composites have greater modulus than neat and alkali-treated hemp/CAB composites (*196*).

Bledzki and coworkers study the effect of enzymatic treatment on the properties of abaca lignocellulose/PP composites in an injection molding process. It is reported that enzymatic treatment removes extractives and amorphous cell wall components, reduces fiber diameter and length and increases thermal decomposition temperatures for abaca lignocellulose. As a result, enzyme-treated abaca lignocellulose/PP composites exhibit 20-45% improvement in moisture resistance, increase in tensile strength and flexural strength by 5-45% and 10-35% respectively. Enzymatic treatment renders the abaca lignocellulose/PP composites better acid and base resistance (*197*).

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c. Chemical Modification

It refers to a chemical process employed to modify the surface reactivity of lignocellulose with the objective of improving the compatibility between lignocellulose and polymers. Chemical modifications commonly used include esterification, etherification, radical condensation, *etc* (*10*, *198*).

(i) Esterification. Acetylation of lignocellulose, a well-known esterification, causes the plasticization of reactive hydroxyl groups and improves the compatibility between lignocellulose and polymeric matrix (*90*, *92–94*, *199*, *200*). It is based on the reaction of hydroxyl groups of lignocellulose with acetic or propionic anhydride at elevated temperatures (*201*), as shown in *eq.* 2.

$$\text{Fiber-OH} + \underset{\text{acetic anhydride}}{CH_3C(=O)\text{-}O\text{-}C(=O)\text{-}CH_3} \rightarrow \underset{\text{acetylated fiber}}{\text{Fiber-O-}C(=O)\text{-}CH_3} + \underset{\text{acetic acid}}{CH_3C(=O)\text{-}OH} \qquad (\textit{eq.2})$$

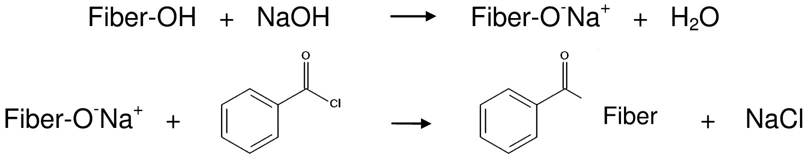
The closely packed structure of lignocellulose hinders the accessibility of acetylation agents to hydroxyl groups and thus slows down the acetylation rate. Wood acetylation has been adopted by the industry to stabilize against moisture, improve dimensional stability and weathering resistance. Naik and coworkers esterify the lignocellulose of banana, hemp and sisal with MA, and study the swelling properties of MA-lignocellulose/HDPE composites. A significant reduction in swelling is observed for MA-lignocellulose/HDPE composites after prolonged exposure to water and steam. MA-sisal/HDPE and MA-hemp/HDPE composites at 40 wt% biofiber loading appear to be optimal, and MA-sisal/HDPE out-performs MA-hemp/HDPE composites in water resistance properties (*202*).

Zhang and coworkers esterify wood lignocellulose with octanoyl chloride and palmitoyl chloride respectively in dimethylformamide (DMF) with intention to improve the compatibility between wood lignocellulose and HDPE. The strong solvation effect of DMF enables high esterification rate. It is found that C-8 acid chloride has higher reactivity than C-16 acid chloride, resulting in a higher degree of esterification. It is also found that C-8 acid chloride esterified wood lignocellulose/HDPE composites at 60 wt% loading show reduction in water absorption, flexural strength and modular compared to neat wood lignocellulose/HDPE composites (*203*).

Botaro and coworkers esterify alkali-treated (2% NaOH solution) sisal lignocellulose using benzophenonetetracarboxylic dianhydride (BTDA). BTDA is chosen for its polar and aromatic groups to intensify the interaction between sisal and phenolic matrix. Reinforcing phenols with 15 wt% of esterified sisal of 3 mm in length improves the hydrophobicity of the sisal/phenol composites at the expense of reduced impact strength (*204*).

(ii) Etherification. Etherification of lignocellulose often leads to new properties for BRC (*205*). Prior to etherification, lignocellulose is treated using alkali to generate charged intermediates that facilitates the nucleophilic addition of etherification agents to lignocellulose (*206*).

Benzoyl chloride has demonstrated its capability in improving lignocellulose and polymer adhesion, and enhancing the strength, water resistance and thermal stability of BRC (*eq.*3). Modifying the lignocellulose of flax and oil palm EFB with benzoyl chloride enhances their compatibility with PE and natural rubber, hence improves the mechanical properties of their composites (*207*, *208*).



(*eq.3*)

Acrylonitrile (CH_2=CH-C≡N)) is also used to modify lignocellulose (*eq.*4). Modification of hemp lignocellulose with acrylonitrile leads to significant improvement in the mechanical properties of hemp lignocellulose/unsaturated polyester composites (*123*).

$$\text{Fiber-OH} + \text{CH}_2\text{=CH-C}\equiv\text{N} \longrightarrow \text{Fiber–OCH}_2\text{CH}_2\text{CN}$$

(*eq.4*)

(iii) Free radicals condensation. Lignocellulose and polymers can be induced to form free radicals, which then condense among themselves to form lignocellulose-reinforced composites bonded via covalent bonds. Organic peroxides (ROOR) and potassium permanganate (MnO_4^-) are common free radical-induction agents. Organic peroxides tend to decompose readily forming free radicals in the form of RO• which then initializes the formation of free radicals on lignocellulose and polymers. One hypothetical mechanism is shown in *eq.*5 (*209*) and experimental supports of this hypothesis can be found in selected publications (*207*, *210*).

$$\begin{aligned} \text{RO - OR} &\longrightarrow 2\text{RO}^{\bullet} \\ \text{RO}^{\bullet} + \text{PE-H} &\longrightarrow \text{ROH} + \text{PE}^{\bullet} \\ \text{RO}^{\bullet} + \text{Fiber-H} &\longrightarrow \text{ROH} + \text{Fiber}^{\bullet} \\ \text{PE}^{\bullet} + \text{Fiber}^{\bullet} &\longrightarrow \text{PE - Fiber} \end{aligned}$$

(*eq.5*)

Compatibilizer

A compatibilizer is a chemical that functions at the interface between the reinforcement and matrix with one end of the molecule tethered to the reinforcement and the other end to the matrix. A compatibilizer is used to improve the mechanical performance and wet-dimensional stability of the BRC (*182*). Recent advancements in this area have been reviewed (*10*, *211*, *212*).

Anhydrides react with the hydroxyl group of lignocellulose forming ester bonds (*213*), and acetic anhydride (AA), MA, succinic anhydride (SA) and phthalic anhydride (PA) are common compatibilizers used in BRC.

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Table VI. Effect of MAPP on the mechanical properties of biofibers/PP composites

			Biofibers/PP Composites		
Biofibers	*Fiber Loading (%)*	*Tensile Strength (MPa)*	*Tensile Modulus (GPa)*	*Flexural Strength (MPa)*	*Flexural Modulus (GPa)*
None	0	32	1.7	41	1.4
Wood	50	39	5.5	68	5.3
Sisal	50	60	6.0	85	5.1
Jute	50	73	8.5	100	7.5

(i) Maleated copolymers. MA is able to react with lignocellulose and polymers, and used as compatibilizer to strengthen the bonding between biofibers and polymers. Grafting PP with MA forms a more effective compatibilizer, MA-grafted-PP, MAPP in short. The anhydride groups of MAPP may react with the surface hydroxyl groups of lignocellulose forming ester bonds whilst the PP tail of MAPP entangles and fuses upon heating (< 200 °C) forming LC-MAPP-polymer composites (*214–218*). MA-grafted-PE, MAPE in short, is also used as a compatibilizer in BRC design. MAPP has proven record in improving the compatibility between a wide range of biofibers, and polymeric matrix (*219–223*), as shown in Table VI.

(ii) Silanes. Silanes have a generic chemical formulae $R_{(4-n)}$-Si-$(R'X)_n$ ($n = 1,2$) where R is alkoxy, X is an organo functional group, and R' is an alkyl group linking the silicon atom and the organo functional group. Typical organic functional groups of the silanes include amino, mercapto, glycidoxy, vinyl and methacryloxy, *etc*. Silanes are first hydrolyzed forming reactive silanol groups which may react with the hydroxyl groups of lignocellulose forming C-O-Si bonds or condense on the surfaces of lignocellulose forming macromolecular network to render lignocellulose with improved hydrophobicity (*224*). The interfacial adhesion between the silanes and polymeric matrix, and the properties of the BRC may be enhanced (*225–227*). The Si-O-C bonds are not stable and can be hydrolyzed forming blockage over surface hydroxyl groups, acting as interfacial enhancement between treated biofibers and polymeric matrices. Trialkoxysilanes are the most common silanes used in the design of BRC.

(iii) Isocyanates. The isocyanate functional group (-N=C=O) of isocyanates is able to react with the hydroxyl groups of lignocelullose, and reduces the hydrophilicity of lignocelullose (*228*). Isocyanates have been used as compatibilizers for BRC (*229*, *230*).

Karmarkar and coworkers develop a novel compatibilizer (*m*-TMI-g-PP) by grafting m-isopropenyl-alpha,alpha-dimethylbenzyl-isocyanate (M-TMI) onto isotactic PP in a twin screw extruder. Applying *m*-TMI-g-PP in the fabrication of wood lignocellulose/PP enhances the tensile strength and the flexural properties of the composites by 45% and 85% respectively in the loading range of 10 to 50

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wt%. The elongation at break and impact strength of the composites so formed are reduced (*231*).

Ashori and coworkers study the compatibilizing effect of polybutadiene isocyanate (PBNCO) on the reinforcement of bagasse lignocellulose on PP in the presence of MAPP. It is found that the interfacial bonding and tensile strength increases and the impact strength of the composites decreases as the fiber loading increases from 10 to 40%, (*232*).

Recent Advancement in BRC

a. Laboratory Innovations

Much of the recent advancement in BRC is around the designing of 100% biocomposites. Zhang and coworkers report that all-lignocellulose composites can be assembled via the grafting of sisal lignocellulose with benzyl groups. By controlling the extent of benzyl substitution, the thermal formability and mechanical properties of the all-lignocellulose composites can be optimized. Filling the all-lignocellulose composites with sisal lignocellulose can enhance their performance (*233*).

Researchers at USDA (*234*) report that the functionality of PLA can be enhanced through the reinforcement effect of sugar beet pulp (SBP) with and without sorbitol and glycerol plasticizer. Increasing SBP loading improves Young's modular and weakens tensile strength of the SBP/PLA composites. Reinforcing PLA with glycerol-plasticized SBP at 40% loading results in a cohesive and continuous composite structure. Their study concludes that reinforcing PLA with biofibers affords 100% biocomposites with improved Young's modular at competitive prices.

Narayan and coworkers developed a starch-vegetable oil copolymer using twin-screw co-rotating extrusion. The starch-vegetable oil grafted copolymer can be reinforced with biofibers in the presence of compatibilizers such as MA in a reactive extrusion process. So-formed resin can be injection molded to produce biodegradable products (*235*).

In an innovative research, a low-cost 100% biocomposite is produced by co-extruding rice flour, water and glycerol. Reinforcing the rice flour/glycerol composite with alkali-treated sisal lignocellulose improves its mechanical properties and moisture resistance, and optimal reinforcement is achieved at 20 wt% loading of 4 mm fiber. Alkylization of sisal lignocellulose improves the tensile strength up to about 4 times. Rice flour-based 100% biocomposites, when synergistically reinforced with modified sisal lignocellulose, may afford promising 100% biocomposites for industry applications (*236*).

Researchers at the University of Maine have developed a composition and a method for making lignocellulose-based engineering composites from reactive polymer, thermal stabilizing agents and lignocellulose (*237*). A method of assembling lignocellulose-based engineering composites from lignocellulose and either of PET, Nylon, ABS or PEEK is patented for proprietary where lubricants

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are added to the compounding chamber to prevent the thermal degradation of lignocellulose (*238–240*).

b. Commercial-Driven Innovations

More than 1.5 million tons of BRC are produced per annum globally, approximately 1 million tons in North America, 200,000 tons in China, 170,000 tons in Europe and 100,000 tons in Japan (*241*). In North American, BRC are found mainly in four markets (i) building and construction industry, as decking, railing, children's playgrounds and window profiles (ii) automobile industry as body parts and interior upholstery (iii) consumer goods as children's toys (iv) miscellaneous as infrastructure materials, as illustrated in Figure 10. The North American BRC industry is purely commercial driven. In Europe, BRC is mainly found in the automobile industry. European BRC industry is driven by EU's environmental regulations that the end-of-life disposal of vehicles must meet a minimum of 85% vehicles' body weight by 2015.

Approximate 5% of the energy consumed in an automobile's lifetime is used for manufacturing and the rest is for moving the vehicle around for a period of 15 to 20 years. Displacing glass fiber with biofibers in BRC automobile parts presents a real business opportunity. In addition to its environmental benefits, lignocellulose is favored for its low specific strength and sound damping properties. Replacing glass fiber with hemp lignocellulose has the potential to reduce about 1.4 kg of CO_2 emission per kilogram of glass fiber replaced throughout entire service life of an automobile (*242*).

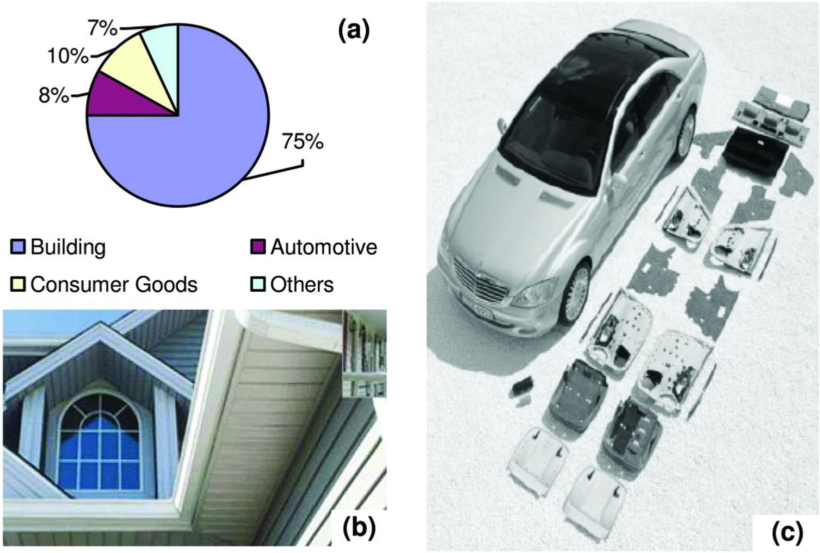

Figure 10. (a) Commercial BRC products in North American Market (b) BRC in building applications, (c) BRC automobile parts. (Reproduced with permission of (243). Copyright Wiley-VCH Verlag GmbH & Co. KGaA)

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Table VII. BRC in automobile applications (*243*)

Auto Makers	*Biofibers*	*Auto Parts*	*Model*
Daimler Chrysler	▪ Flax ▪ Hemp ▪ Sisal	▪ door cladding ▪ seatback linings ▪ package shelves	Mercedes Benz Class A, C, E, S
	▪ Coconut ▪ Caoutchouc	▪ seat bottom ▪ back cushions ▪ head restraints	
	▪ Abaca	▪ under-floor body panels	
	▪ Castor oil seed	▪ flexible tubing for fuel ▪ brake systems	
BMW	▪ Flax ▪ Sisal	▪ interior door linings & panels	3,5,7 Series
	▪ Cotton	▪ soundproofing	
	▪ Wool	▪ upholstery	
	▪ Wood fiber	▪ seatback cushions	
Toyota	▪ Kenaf	▪ package shelves ▪ body structure	Lexus i-foot/i-unit
GM	▪ Kenaf/flax mixture ▪ Wood fiber	▪ package trays ▪ door panel inserts ▪ seatbacks ▪ cargo area floor	Satum, L300s Opel, Vectras Cadillac DeVille, GMC Envoy, Chevrolet TrailBlazer
Honda	▪ Wood fiber	▪ cargo area floor	Pilot SUV
Ford	▪ Corn	▪ goodyear tires	Fiestas
	▪ Wood fiber	▪ sliding door inserts	Ford, Freestar

Since the 1930s when the first BRC automobile part was invented by Henry Ford, BRC automobile parts have grown wide-ranging (Table VII). Lignocellulose of flax, sisal and hemp is processed into door cladding, seatback linings and floor panels. Coconut lignocellulose is used to make seat bottoms, back cushions and head restraints. Cotton lignocellulose is used to provide sound proofing and wood lignocellulose is used in seatback cushions. Abaca is used in underfloor mats. The BMW Group incorporates about 10,000 tons of lignocellulose into their vehicles per annum. DaimlerChrysler champions the sustainable vehicle movement and has launched about 50 biocomposite automobile parts in European market alone.

PP is a preferred thermoplastic resin for automobile BRC due to its low density, excellent processability, appropriate mechanical properties and electrical properties, good dimensional stability and impact strength (*229*). Common thermoset resins used include unsaturated polyester and vinylester due to their polarity. Orthophthalic polyester yields rigid BRC with low heat resistance, isophthalic polyester yields moisture resistant BRC and vinylester yields tough BRC comparing to polyesters. Epoxy resins yield high performance and weather-resistance BRC, however their adoption is challenged by long curing time and high cost (*244*).

Among the BRC manufacturing processes, reactive extrusion provides a conducive environment for polymeric matrix, reinforcement fibers and compatibilizers to assemble, however, high shear of the extrusion process causes damages on lignocellulose fiber (loss of strength and changes in aspect ratio and distribution). Drzal and coworkers patent a proprietary process, known as Biocomposite Stampable Sheet Process (BCSSS) (*245*). This process enables good fiber dispersion at minimal damage, flexible formulations and inclusion of a variety of biofibers with the aid of electrical alignment and powder impregnation technology. The invention enables assembly of performance biocomposites from biofibers and unsaturated polyester resin or bioresins such as PLA.

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Thoughts on Biofiber Composite Design

A Bioinspired Design Strategy

Natural material is often small with built-in intelligence that senses and responses to external changes and feedbacks in order to optimize its own system for better survival (*246*). A bioinspired design strategy is drawn from the understanding of nature and learns how to build things by mimic the chemistry and structure of natural biomaterials in order to deliver superior functionality with minimum impact. Inspiring examples of nature at work are all around us. A sea cucumber, like other echinoderms, has the ability to rapidly and reversibly alter the stiffness of its inner dermis which creates considerable survival advantages (*247*). By mimic the chemo-responsive nature of the sea cucumber dermis, Wedder and coworkers design a new family of CNs-reinforced morphing biocomposites by controlling the hydrogen bonds among CNs, as shown in Figure 11 (*248*, *249*).

The similarities between many natural materials such as teeth, bones, egg shell and diatoms are pronounced in that they are all inorganic/organic hybrid composites composed of a biopolymer matrix (*i.e.* proteins, polysaccharides) and a mineral reinforcement (*i.e.* hydroxyapatite, calcuim carbonate). The matrix component imparts toughness and resilience while the reinforcement renders strength, hardness and stiffness (*250*). Cotton cellulose is a preferred textile fiber for apparel use for reasons such as comfort and feel. A major disadvantage of cellulose is flammability and low strength. Delhom and coworkers have developed flame-retardant inorganic/organic hybrid nanocomposites from regenerated cellulose of cotton and nanoparticles of layered silicate, montmorillonite. The hybrid nanocomposites show significant improvement in thermal properties and tensile strength. The degradation temperature of the composites increases by 45 °C and the tensile strength increases by about 80% comparing to cotton cellulose (*251*).

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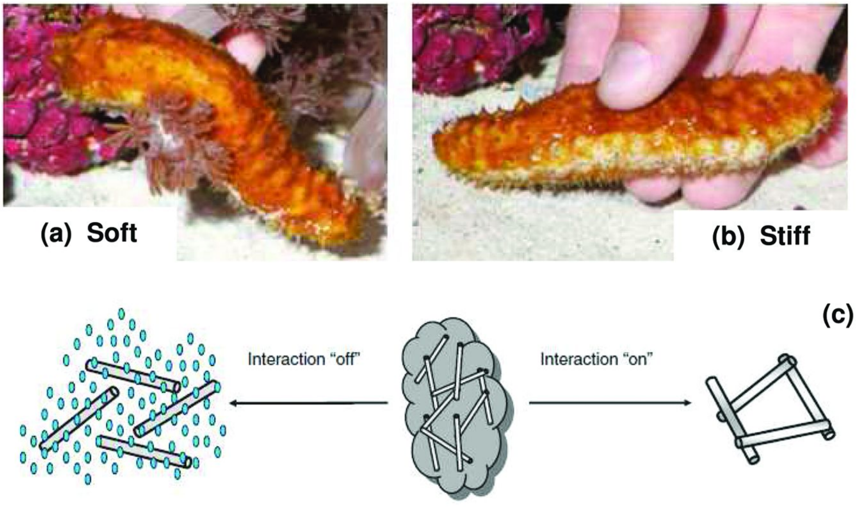

Figure 11. (a) Natural soft dermis of sea cucumber (b) Stiffened dermis at sense of "risk" (c) "Switching" H-bonds among CNs is the key of morphing biocomposite. (Reproduced with kind permission of (248). Copyright 2008 Science.)

Natural biomaterials have characteristic hierarchical structure. The precise way a composite material is organized at the microscopic level determines whether the material is hard and resilient like enamel which resists cracking; or hard and brittle like eggshell which cracks easily. A synergism renders the composites properties unavailable from individual components, while the wide variety of matrix and reinforcing materials allows designers to resort on an optimum combination.

Holistic Thinking

Life cycle assessment (LCA) provides a system approach for assessing the potential impacts of a product by looking at its whole lifecycle from raw materials acquisition, use and to the products end-of-life disposal including recycling, incinerations, landfill, and composting (*252*). The LCA of hemp- and flax-reinforced composites for automobile parts has been studied (*253–255*). Wötzel and coworkers compared the LCA data of an Audi A3 part made of ABS co-polymer with that of a hemp/epoxy composite. It is found that the production of hemp/epoxy composite consumes no more than 45% energy and emitted less GHG comparing to the ABS copolymer. The study suggests that incineration could be a good end-of-life option for the hemp/epoxy composite as hemp is carbon-neutral. The study also reveals that hemp/epoxy composites discharge higher level of nitrates and phosphates, and emit more NO$_x$ than ABS copolymer. The finding is believed to relate to the use of fertilizer during reed cultivation.

Researchers at the Federal Institute of Technology Switzerland conduct LCA for a glass fiber (42 wt%)/PP and a china reed (53 wt%)/PP composites. Their study reveals that eco-indicator 95 of the China reed/PP composite on carcinogenicity and acidification is 63% that of the glass fiber/PP composite. The production process of the china reed/PP composite releases less GHG and NO_x gases, and discharges less nitrates than those of the glass fiber/PP composite. Similarly, the China reed/PP composite discharges more phosphates (1.67g) comparing to the glass fiber/PP composite (0.59g). The finding is believed relating to the use of fertilizer during reed cultivation (*256*).

The energy used for hemp and flax production is less than 10% of the energy used for PP fiber production, and is about 15% when the use of fertilizers is considered (*257*). The energy used to produce flax/PP composites is about 20% that of glass/PP composites (9.6 MJ/kg vs 54.7 MJ/kg) (*258*). When biofiber composites are produced with high fiber loading for equivalent performance the amount of synthetic polymers such as PP can be reduced, hence, total energy consumption is reduced (*255*).

The magnitude of the environmental benefits for a biocomposite is a sum of the total environmental credits throughout a material's lifecycle. Biofiber can be considered as being carbon-neutral as it releases the same amount of CO_2 during incineration as what's sequestered during its growth. In order to account for it in balance throughout the life cycle, it is necessary to identify an appropriate disposal method for the product at the end of service life. This would help avoid disposing a biomaterial in a non-controlled anaerobic environment causing CH_4 emission with greater greenhouse impact on the atmosphere. After all, a true biofiber-reinforced composite should be performance-sound and competitive to deliver its intended purpose at no expense of nature.

An Inter-Disciplinary Approach

Lignocellullose replacing glass and carbon fibers offers many advantages due to its light weight, low cost, good specific properties, low processing energy, carbon-neutral, biodegradable, and ease of recycling. However, attempts to design high performance lignocellulose-reinforced composites are challenged by several technical challenges, some of which include chemical compatibility, high moisture uptake and thermal degradation.

The availability of nano-sized zinc oxide, silver, titanium oxide and even nano clays opens new possibilities to engineer nanocomposites with properties matching the requirement of applications (*259*). Marques and coworkers have developed a method for making high opacity paper containing 46% TiO_2. The TiO_2-paper is prepared through acidic hydrolysis of titanyl sulphate in the presence of cellulose. It is found that cellulose promotes the nucleation and growth of TiO_2 nanoparticles, yielding a well dispersed hybrid inorganic/organic composite (*260*).

Lignocellulosic composites have low degradation temperature and are susceptible to moisture. One promising approach to overcome these is through chemical modification of the hydroxyl groups of exposed cellulose. In a specific study, tetraethyl orthosilicate is used as silica precursor and heterpoly acids as catalyst to modify cellulose. Under optimal conditions, about 40-60% of silica is found deposited on the surface of cellulose in the form of a thin discontinuous film or as nano/meso particles via non-covalent bonds. Silica/cellulose hybrid

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composites exhibit improved hydrophobicity, wet-dimension stability, thermal stability, thermal insulation properties and bending strength (*261–263*).

Electrospinning (ELSP) has attracted considerable interest due to its versatility in producing fiber nanocomposites from materials of diverse origins. The electrospun nanofiber composites have found applications in widespread areas including conducting nanofibers, drug delivery, sensors, protective clothing, cosmetics, *etc*. Recent progress in the ELSP processing technology and its applications in the assembly of new fiber composites is reviewed (*149*). There have been an increasing number of reports on the preparation of electrospun nanocomposite fibers containing inorganic nanofillers such as nanosilicate, calcium carbonate, hydroxyapatite and silver nanoparticles (*264–267*).

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Closing Remark

The renewed interest in biofibers is spurred by growing appreciation of sustainable development. Biofibers have found widespread applications, lignocellulose and cellulose being mostly used as reinforcement fillers for biocomposites, cellulose and regenerated cellulose in non-woven, and cellulose nanocrystallites as nanofiller for functional nanocomposites (*268*). Biofibers are preferred over synthetic and mineral fillers due to their light weight, specific mechanical properties, biodegradability, abundance and low cost. The hydrophilic nature of biofibers disadvantages them from deliverance of target performance, and a common remedy adopted has been chemical modification. Acetylation of lignocellulose and cellulose provides a cost effective solution to reduce the susceptibility of biofibers to moisture, fungi and weathering attack (*96*) at the expense of loss of biodegradation.

Heat treatment of lignocellulose changes its chemical composition and surface polarity. It plasticizes lignocellulose hence enabling auto-adhesion among lignocellulosic fibers to take place. Laccase-assisted adhesion coupled with heat treatment provides a sustainable approach towards binderless biocomposite products. It is likely to remain the subject of intensive investigations for commercial exploitation (*80*).

Introducing lignocellulose or cellulose into an inorganic matrix enables the assembly of inorganic/organic hybrid composites. This is to leverage on the advantage of polar and hydrophilic characteristics of biofibers instead of depriving it. The inorganic/organic hybrid composites may deliver surprising functionality by integrating the low density, toughness and flexibility of biofibers and the hardness, strength and heat resistance of inorganic components. The key towards successful hybridization lies on the chemcial compability and interfacial adhesion between the inorganic and biofibers, and an assembly process enabling it to happen at minimal energy and waste (*269*).

Incorporating biofibers into biopolymers provides it with better mechanical properties and avails greater variety of biodegradable products at affordable price. It renders biopolymers ready for non-structural applications, displacing fossil-based products.

Nature builds lignocelluloses from CO_2 and H_2O and has programmed to recycle it in a timely way to the starting chemicals through biological, mechanical and chemical means. Challenge remains to be how to honor the perfect hierarchical structure of lignocellulose and be inspired by its intrinsic attributes in formulating a biocomposite design strategy so that a total "destruction-reassembly" approach can be avoided.

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References

1. Sierra, R.; Smith, A.; Granda, C.; et al. *Chem. Eng. Prog.* **2008**, *104* (8), S10–S18.
2. Zhang, Y-H. P.; Lynd, L. R. *Biotechnol. Bioeng.* **2004**, *88*, 797–824.
3. Ding, S. Y.; Himmel, M. E. *J. Agric. Food Chem.* **2006**, *54* (3), 597–606.
4. Micic, M.; Radotic, K.; Jeremic, M.; et al. *Macromol. Biosci.* **2003**, *3*, 100–106.
5. Mohanty, A. K.; Misra, M.; Hinrichsen, G. *Macromol. Mater. Eng.* **2000**, *276/277*, 1–24.
6. Rubin, E. M. *Nature* **2008**, *454*, 841–845.
7. Shchniewind, A. P. *Concise Encyclopedia of Wood & Wood-Based Materials*; Pergamon: Oxford, 1989.
8. Karnani, R.; Krishnan, M; Narayan, R. *Polym. Eng. Sci.* **1997**, *37* (2), 476–487.
9. Marti-Ferrer, F.; Vilaplana, F.; Ribes-Greus, A.; et al. *J. Appl. Polym. Sci.* **2006**, *99* (4), 1823–1831.
10. Li, X.; Tabil, L. G.; Panigrahi, S. *J Polym. Environ.* **2007**, *15*, 25–33.
11. Yao, F.; Wu, Q. L.; Lei, Y.; et al. *Ind. Crops Prod.* **2008**, *28* (1), 63–72.
12. Kozłowski, R.; Władyka-Przybylak, M. *Polym. Adv. Technol.* **2008**, *19* (6), 446–453.
13. John, M. J.; Anandjiwala, R. D. *Polym. Compos.* **2008**, *29* (2), 187–207.
14. Suizu, N.; Uno, T.; Goda, K. *J. Mater. Sci.* **2009**, *44*, 2477–2482.
15. Reddy, N.; Yang, Y. Q. *Trends BioTechnol.* **2005**, *23* (1), 22–27.
16. Crawford, R. L. In *Lignin Biodegradation and Transformation*; John Wiley and Sons: New York, 1981; ISBN 0471057436.
17. Atalla, R. H.; VanderHart, D. L. *Science* **1984**, *223*, 283–285.
18. Nishiyama, Y.; Langan, P.; Chanzy, H. *J. Am. Chem. Soc.* **2002**, *124*, 9074–9082.

Downloaded by OHIO STATE UNIV LIBRARIES on June 17, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch013

19. Jarvis, M. *Nature* **2003**, *426*, 611–612.
20. Callihan, C.; Clemme, J. In *Microbial Biomass*; Rose, A., Ed.; Academic Press: New York, 1979; pp 271.
21. Ross, P.; Mayer, R.; Benziman, M. *Microbiol. Rev.* **1991**, *55* (1), 35–58.
22. Ciechańska, D.; Struszczyk, H; Kazimierczak, J.; et al. *Fibres Text. East. Eur.* **2002**, *10:1* (36), 27–30.
23. Colvin, J. R.; Leppard, G. G. *Can. J. Microbiol.* **1977**, *23*, 701–709.
24. White, D. G.; Brown, R. M. J. In *Cellulose and Wood-Chemistry and Technology*; Schuerech, C., Ed.; John Wiley and Sons: New York, 1989.
25. Williams, W. S.; Cannon, R. E. *Appl. Environ. Microbiol.* **1989**, *55*, 2448–2452.
26. Brown, R. M., Jr. In *Cellulose: Structural and Functional Aspects*; Kennedy, J. F., Philips, G. O., Williams, P. A., Eds.; Ellis Horwood Ltd: Chirchester, 1989; pp 145−151.
27. Yamanaka, S.; Watanabe, K.; Kitamura, N.; et al. *J. Mater. Sci.* **1989**, *24*, 3141–3145.
28. Gindl, W.; Keckes, J. *Compos. Sci. Technol.* **2004**, *64*, 2407–2413.
29. Yano, H.; Sugiyama, J.; Nakagaito, A. N.; et al. *Adv. Mater.* **2005**, *17*, 153–155.
30. Nakagaito, A. N.; Iwamoto, S.; Yano, H. *Appl. Phys. A: Mater. Sci. Process.* **2005**, *80* (1), 93–97.
31. Ifuku, S.; Nogi, M.; Abe, K.; et al. *Biomacromolecules* **2007**, *8*, 1973–1978.
32. Fontana, J. D.; Joerke, C. G.; Baron, M.; et al. *Appl. Biochem. Biotechnol.* **1997**, *63-65*, 327–328.
33. Wan, Y. Z.; Hong, L.; Jia, S. R.; et al. *Compos. Sci. Technol.* **2006**, *66*, 1825–3182.
34. Wan, Y. Z.; Huang, Y.; Yuan, C. D.; et al. *Mater. Sci. Eng. C.* **2007**, *27*, 855–864.
35. Czaja, W. K.; Young, D. J.; Kawecki, M.; et al. *Biomacromolecules* **2007**, *8*, 1–12.
36. Araki, J.; Kuga, S. *Langmuir* **2001**, *17*, 4493–4496.
37. Habibi, Y.; Goffin, A. L.; Schiltz, N.; et al. *J. Mater. Chem.* **2008**, *18*, 5002–5010.
38. Hanley, S. J.; Revol, J.-F.; Godbout, L.; et al. *Cellulose* **1997**, *4*, 209–220.
39. Helbert, W.; Cavaille, J. Y.; Dufresne, A. *Polym. Compos.* **1996**, *17*, 604–611.
40. Araki, J.; Wada, M.; Kuga, S.; Okano, T. *J. Wood Sci.* **1999**, *45*, 258–261.
41. Beck-Candanedo, S.; Roman, M.; Gray, D. G. *Biomacromolecules* **2005**, *6*, 1048–1054.
42. Angles, M. N.; Dufresne, A. *Macromolecules* **2001**, *34*, 2921–2931.
43. Hanley, S. J.; Revol, J. F.; Godbout, L.; et al. *Cellulose* **1997**, *4*, 209–220.
44. Elazzouzi-Hafraoui, S.; Nishiyama, Y.; Putaux, J. L.; et al. *Biomacromolecules* **2008**, *9*, 57–65.
45. Nishino, T.; Takano, K.; Nakamae, K. *J. Polym. Sci. B.* **1995**, *33*, 1647–1751.
46. Sturcova, A.; Davies, G. R.; Eichhorn, S. J. *Biomacromolecules* **2005**, *6*, 1055–1061.
47. Hsieh, Y. C.; Yano, H.; Nogi, M.; et al. *Cellulose* **2008**, *15*, 507–513.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch013

48. Eichhorn, S. J.; Baillie, C. A.; Zafeiropoulos, N.; et al. *J. Mater. Sci.* **2001**, *36* (9), 2107–2131.
49. Shibata, M.; Oyamada, S.; Kobayashi, S.; et al. *J. Appl. Polym. Sci.* **2004**, *92*, 3857–3863.
50. Revol, J. F.; Bradford, H.; Giasson, J.; et al. *Int. J. Biol. Macromol* **1992**, *14*, 170–172.
51. Araki, J.; Wada, M.; Kuga, S.; Okano, T. *Colloids Surf., A* **1998**, *142*, 75–82.
52. Henriksson, M.; Henriksson, G.; Berglund, L. A.; et al. *Eur. Polym. J.* **2007**, *43*, 3434–3441.
53. Mo, X. Q.; Cheng, E. Z.; Wang, D. H; et al. *Ind. Crops Prod.* **2003**, *18* (1), 47–53.
54. Averous, L.; Le Digabel, F. *Carbohydr. Polym.* **2006**, *66* (4), 480–493.
55. Schirp, A.; Loge, F. J.; Englund, K. R.; et al. *For. Prod. J.* **2006**, *56* (10), 90–96.
56. Youngquist, J. A. *Wood Handbook - Wood as an Engineering Material*; USDA Forest Products Laboratory: USA, 1999; pp 10-1−10-31.
57. Sellers, T. *For. Prod. J.* **2001**, *51*, 12–22.
58. Smith, D. C. *For. Prod. J.* **2004**, *54*, 8–17.
59. Grigoriou, A. H. *Wood Sci. Technol.* **2000**, *34* (4), 355–365.
60. Mo, X. Q.; Wang, D.; Sun, X. S. In *Natural Fibers, Biopolymers, and Biocomposites*, Mohanty, A. K., Misra, M., Drzal, L. T., Eds.; Taylor & Francis: FL, 2005; pp 875.
61. Xing, C.; Zhang, S. Y.; Deng, J.; et al. *Wood Sci. Technol.* **2006**, *40*, 637–646.
62. Hervillard, T.; Cao, Q.; Laborie, M. P. G. *Bioresearch* **2007**, *2* (2), 148–156.
63. Halvarsson, S.; Edlund, H.; Norgren, M. *Ind. Eng. Chem. Res.* **2010**, *49* (3), 1428–1435.
64. Hoareau, W.; Oliveira, F. B.; Grelier, S.; et al. *Macromol. Mater. Eng.* **2006**, *291* (7), 829–839.
65. Li, K. C.; Geng, X. L. U.S. Patent Appl. 10/802,500, 2002.
66. Rivers, J. D.; Griffin, B. J.; Hagiopol, C.; et. al. U.S. Patent Appl. 10/849,558, 2005.
67. Li, K. C. U.S. Patent Appl. 11/372,998, 2006.
68. Li, K. C.; Liu, Y. U.S. Patent 7,393,930, 2008.
69. Li, K. C. U.S. Patent 7,785,440, 2010.
70. Bouajila, J.; Limare, A.; Joly, C.; et al. *Polym. Eng. Sci.* **2005**, *45* (6), 809–816.
71. Xu, J. Y.; Widyorini, R.; Yamauchi, H.; et al. *J. Wood. Sci.* **2006**, *52* (3), 236–243.
72. Rowell, R. M.; McSweeny, J. D. *Mol. Cryst. Liq. Cryst.* **2008**, *483*, 307–325.
73. Esteves, B. M.; Pereira, H. M. *Bioresearch* **2009**, *4* (1), 370–404.
74. Mayer, A. M.; Staples, R. C. *Phytochem.* **2002**, *60*, 551–565.
75. Felby, C.; Pedersen, L. S.; Nielsen, B. R. *Holzforschung* **1997**, *51*, 281–286.
76. Felby, C.; Hassingboe, J.; Lund, M. *Enzyme Microb. Technol.* **2002**, *31*, 736–741.
77. Felby, C.; Thygesen, L. G.; Sanadi, A.; et al. *Ind. Crops Prod.* **2004**, *20*, 181–189.

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

78. Kharazipour, A. *J. Adhes. Sci. Technol.* **1997**, *11*, 419–427.
79. Widsten, P.; Laine, J. E.; Tuominen, S.; et al. *J. Adhes. Sci. Technol.* **2003**, *17*, 67–78.
80. Widsten, P.; Kandelbauer, A. *Biotechnol. Adv.* **2008**, *26*, 379–386.
81. Widsten, P.; Kandelbauer, A. *Enzyme. Microb. Technol.* **2008**, *42* (4), 293–307.
82. Cao, Y.; Duan, X.; et al. *Proc. China Assoc. Sci. Technol.* **2007**, *3*, 114–118.
83. Kharazipour, A.; Hüttermann, A.; Kühne, G.; et. al. European Patent Appl. EP05,65,109, 1993.
84. Qvintus-Leino, P.; Widsten, P.; Tuominen, S.; et. al. Int. Patent Appl. WO03047826, 2003.
85. Hüttermann, A.; Nonninger, K.; Kharazipour, A. Int. Patent Appl. WO9,831,729, 1998.
86. Viikari, L.; Hase, A.; Qvintus-Leino, P.; et. al.. Int. Patent Appl. WO9831763, 1998.
87. Viikari, L.; Hase, A.; Qvintus-Leino, P.; et. al. Int. Patent Appl. WO9,831,764, 1998.
88. Hon, D. N-S. *Chemical Modification of Wood materials*; Marcel Dekker: New York, 1996; pp 370.
89. Hill, C. *Wood Modification: Chemical, Thermal and Other Processes*; John Wiley and Sons: WS, England; 2006; pp 239.
90. Rowell, R. M. In *Handbook of Wood Chemistry and Wood Composites* Rowell, R. M., Ed; Taylor and Francis: FL, 2005; pp 381−420.
91. Rowell, R. M.; Tillman, A. M.; Simonson, R. *J. Wood Chem. Technol.* **1986**, *6* (3), 427–448.
92. Nelson, H. L.; Richards D. I.; Simonson, R. European Patent 650,998, 1994.
93. Nelson, H. L.; Richards, D. I. Simonson, R. European Patent 746,570, 1995.
94. Nelson, H. L.; Richards, D. I. Simonson, R. European Patent 799,272, 1995.
95. Murray, J. E. PCT GB1997/001,858, 1997.
96. Rowell, R. M. *For. Prod. J.* **2006**, *56* (9), 4–12.
97. Gomez-Bueso, J.; Westin, M.; Torgilsson, R.; et al. *Holz. Roh- Werkst.* **2000**, *58* (1−2), 9–14.
98. Korai, H.; Kiguchi, M.; Hosoya, S.; et al. *J. Wood Sci.* **2001**, *47*, 24–29.
99. Östenson, M.; Gatenholm, P. *Langmuir* **2005**, *21* (1), 160–165.
100. Laufenberg, T. L. In *Paper and Composites from Agro-Based Resources*; Rowell, R. M., Young, R. A., Rowell, J. K., Eds.; CRC Lewis Publishers: FL, 1996; pp 337−350.
101. Setterholm, V. C. *Tappi. J.* **1985**, *68* (6), 40–42.
102. Hunt, J. F.; Gunderson, D. E. *Tappi Proceedings of the 198th Corrugated Containers Conference*; Tappi Press: USA, 1988; pp 11−17.
103. Scott, C. T.; Laufenberg, T. L. *Proceedings of the Pacific Timber Engineering Conference*; TRADA 632; 1994; pp 2.
104. Sanadi, A. R., Rowell, R. M.; Young, R. A. In *Materials Interaction Relevant to Recycling of Wood-Based Materials*; Rowell, R. M., Laufenberg, T. L, Rowell, J. K., Eds.; 1992; Vol. 266, pp 81−92.
105. Xu, Y.; Lee, M. C. U.S. Patent Appl. 12/396,193, 2009.
106. Gerault; P. U.S. Patent Appl. 7/101,076, 1987.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch013

107. Tan, M.; Noguchi, T.; Kawano, M.; et. al. U.S. Patent 5,785,817, 1998.
108. Hasegawa, O.; Ohno, Y. U.S. Patent 6,421,957 B1, 2002.
109. Xu, Y.; Lee, M. C. U.S. Patent Appl. 12/538,726, 2009.
110. Frederiksen, J. H. European Patent EP1,840,043, 2009.
111. Tan, M.; Noguchi, T.; Kawano, M.; et. al. U.S. Patent 5,785,817, 1998.
112. Hasegawa, O.; Ohno, Y. U.S. Patent 6,421,957 B1, 2002.
113. Hollenberg, D. H.; Manning, J. H.; Martin, R. L.; et. al. U.S. Patent 4,822,452, 1989.
114. Dahiya, A.; Kamath, M. G.; Hegde, R. R. http://www.engr.utk.edu/mse/Textiles/Wet%20Laid%20Nonwovens.htm.
115. Wood, D. E. U.S. Patent 3,972,092, 1976.
116. Wolff, H.; Byrd, V. L. *Tappi J.* **1990**, *73* (9), 159–161.
117. Hansen, M. R.; Young, R. H., Sr. U.S. Patent 5,352,480, 1994.
118. Wierer, K. A.; Poths, H.; Goetze, R. U.S. Patent 6,458,299, 2002.
119. Paulapuro, H. *Papermaking Science and Technology*; Fapet Oy: Finland, 2000; pp 95−98, ISBN 9525216187.
120. Stevens, D. R.; Eagen, B.; Boyd, S. G. U.S. Patent 5,804,262, 1998.
121. Sain, M. M.; Behzad, T. U.S. Patent Appl. 2005/0,245,161A1, 2005.
122. Thielemans, W.; Wool, R. P. *Polym. Comps.* **2005**, *26* (5), 695–705.
123. Mehta, G.; Drzal, L. T.; Mohanty, A. K.; et al. *J. Appl. Polym. Sci.* **2006**, *99* (3), 1055–1068.
124. Laszlo, J. A.; Dintzis, F. R. *J. Appl. Polym. Sci.* **1994**, *52*, 521–528.
125. Waiss, A. C.; Wiley, M. E.; Kuhnle, J. A.; et al. *J. Environ. Qual.* **1973**, *2*, 369–371.
126. Masri, M. S.; Reuter, F. W.; Friedman, M. *J. Appl. Polym. Sci.* **1974**, *18*, 675–681.
127. Randall, J. M. *For. Products J.* **1977**, *27* (11), 51–56.
128. Bhattacharyya, A. K.; Venkobachar, C. *J. Environ. Eng.* **1984**, *110*, 110–122.
129. Phalman, J. E.; Khalafalla, J. E. *Use of Ligochemicals and Humic Acids to Remove Heavy Metals from Process Waste Streams*; U.S. Department of Interior, Bureau of Mines, 1988, RI 9200.
130. Verma, K. V. R.; Swaminathan, T.; Subrahmnyam, P. V. R. *J. Environ. Sci. Health* **1990**, *A25* (2), 242–265.
131. Shukla, S. R.; Sakhardande, V. D. *J. Appl. Polym. Sci.* **1991**, *42*, 825–829.
132. Maranon, E.; Sastre, H. *React. Polym.* **1992**, *18*, 172–176.
133. Lalvani, S. B.; Wiltowski, T. S.; Murphy, D.; et al. *Environ. Technol.* **1997**, *18* (11), 1163–1168.
134. Vaughan, T.; Seo, C. W.; Marshall, W. E. *Bioresour. Technol.* **2001**, *78*, 133–139.
135. Basso, M. C.; Cerrella, E. G.; Cukierman, A. L. *Ind. Eng. Chem. Res.* **2002**, *41* (15), 3580–3585.
136. Reddad, Z.; Gerente, C.; Andres, Y.; et al. *Carbohydr. Polym.* **2002**, *49*, 23–31.
137. English, B. In *Paper and Composites from Agro-Based Resources*; Rowell, R. M., Young, R. A., Rowell, J. K., Eds.; CRC Lewis Publishers: FL, 1996; pp 403−426.
138. Shin, E. W.; Han, J. S.; Min, S. H. *Environ. Technol.* **2004**, *25* (2), 185–191.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch013

139. Huang, X. F.; Lim, T. T. *Desalination* **2006**, *190* (1-3), 295–307.
140. Marshall, W. E.; Akin, D. E.; Wartelle, L. H.; et al. *Ind. Crops Prod.* **2007**, *26* (1), 8–13.
141. Kubinsky, E. *Holzforschung* **1971**, *25* (3), 78–83.
142. Tiemann, K. J.; Gardea-Torresdey, J. L.; Gamez, G.; et al. *Enviro. Sci. Technol.* **1999**, *33*, 150–154.
143. Kubinsky, E.; Ifju, G. *Wood Sci.* **1973**, *6* (1), 87–94.
144. Kubinsky, E.; Ifju, G. *Wood Sci.* **1974**, *7* (2), 103–110.
145. Hogue, C. *Chem. Eng. News*, (March 2000, Clearing the Water), pp 31−33.
146. Hogue, C. *Chem. Eng. News*, (August 2000, Muddied Waters), pp 19−20.
147. Herrmann, A. S.; Nickel, J.; Riedel, U. *Polym. Degrad. Stab.* **1998**, *58*, 251–261.
148. Fowler, P. A.; Hughes, J. M.; Elias, R. M. *J. Sci. Food Agric.* **2006**, *86*, 1781–1789.
149. John, M. J.; Thomas, S. *Carbohydr. Polym.* **2008**, *71*, 343–364.
150. Satyanarayana, K. G.; Arizaga, G. G. C.; Wypych, F. *Prog. Polym. Sci.* **2009**, *34*, 982–1021.
151. Thomas, S.; Pothan, L. A.; Cherian, B. M. *Int. J. Mater. Prod. Technol.* **2009**, *36* (1-4), 317–333.
152. Cantwell, W. J.; Morton, J. *Compos.* **1991**, *22* (5), 347–362.
153. University of Cambridge, Department of Engineering. http://www-materials.eng.cam.ac.uk/mpsite/short/OCR/ropes/default.html.
154. Li, H.; Sain, M. M. *Polym.-Plast. Technol. Eng.* **2003**, *42* (5), 853–862.
155. Sgriccia, N.; Hawley, M. C.; Misra, M. *Composites, Part A* **2008**, *39* (10), 1632–1637.
156. Li, S. M.; Ren, J.; Yuan, H.; et al. *Polym. Int.* **2010**, *59* (2), 242–248.
157. Venkateshwaran, N.; Elayaperumal, A. *J. Reinf. Plast. Compos.* **2010**, *29* (15), 2387–2396.
158. Joseph, S.; Sreekala, M. S.; Thomas, S. *J. Appl. Polym. Sci.* **2008**, *110* (4), 2305–2314.
159. Haneefa, A.; Bindu, P.; Aravind, I.; et al. *J. Compos. Mater.* **2008**, *42* (15), 1471–1489.
160. Knothe, J.; Rebstock, K.; Schloesser, T. *3rd International Wood and Natural Fibre Composites Symposium*, Kassel, Germany, 2000; pp B1−12.
161. Hintermann, M.*Conference RIKO-2005*, Hannover, Germany, 2005; pp 21−26.
162. Huda, M. S.; Drzal, L. T.; Mohanty, A. K.; et al. *Compos. Interface* **2008**, *15* (2−3), 169–191.
163. Siregar, J. P.; Sapuan, S. M.; Rahman, M. Z. A.; et al. *J. Food Agric. Environ.* **2010**, *8* (2), 1103–1108.
164. Tay, G. S.; Zaim, J. M.; Rozman, H. D. *J. Appl. Polym. Sci.* **2010**, *116* (4), 1867–1872.
165. Beijing Forestry and Parks Department of International Cooperation, www.bfdic.com/en/Features/Features/79.html.
166. Arif, M. F.; Megat-Yusoff, P. S. M.; Ahmad, F. *J. Reinf. Plast. Compos.* **2010**, *29* (14), 2105–2118.

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Downloaded by OHIO STATE UNIV LIBRARIES on June 17, 2012 | http://pubs.acs.org
Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch013

167. Mohanty, A. K.; Misra, M.; Drzal, L. T. *Compos. Interfaces* **2001**, *8*, 313–343.
168. Herrera-Franco, P. J.; Valadez-Gonzalez, A. *Composites, Part B* **2005**, *36* (8), 597–608.
169. Kalia, S.; Kaith, B. S.; Kaur, I. *Polym. Eng. Sci.* **2009**, *49* (7), 1253–1272.
170. Hendriks, A. T. W. M.; Zeeman, G. *Bioresour. Technol.* **2009**, *100*, 10–18.
171. Xie, Y. J.; Hill, C. A. S.; Xiao, Z. F.; et al. *Composites, Part A* **2010**, *41* (7), 806–819.
172. Nakagaito, A. N.; Yano, H. *Appl. Phys. A: Mater. Sci. Process.* **2004**, *78* (4), 547–552.
173. Palmowski, L.; Muller, J.*II International Symposium on Anaerobic Digestion of Solid Waste*, Barcelona, 1999; Vol. 1, pp 137−144.
174. Mutje, P.; Girones, J.; Lopez, A.; et al. *J. Reinf. Plast. Comp.* **2006**, *25* (3), 313–327.
175. Rowell. R. M. In *International Encyclopedia of Composites*; Lee, S. M., Ed.; VHC: New York, 1991; p 4.
176. Bobleter, O. *Prog. Polym. Sci.* **1994**, *19*, 797–841.
177. Garrote, G.; Dominguez, H.; Parajo, J. C. *Holz. Roh.- Werkst.* **1999**, *57*, 191–202.
178. Paul, S. A.; Piasta, D.; Spange, S.; et al. *Biomicrol.* **2008**, *9* (7), 1802–1810.
179. Renneckar, S.; Zink-Sharp, A.; Glasser, W. G. *Wood Fiber Sci.* **2006**, *38* (3), 427–438.
180. Saha, P.; Manna, S.; Chowdhury, S. R.; et al. *Bioresour. Technol.* **2010**, *101*, 3182–3187.
181. Ibrahim, M. M.; Dufresne, A.; El-Zawawy, W. K.; et al. *Carbohydr. Polym.* **2010**, *81* (4), 811–819.
182. Bledzki, A. K.; Gassan, J. *Prog. Polym. Sci.* **1999**, *24*, 221–274.
183. Ray, D.; Sarkar, B. K.; Rana, A. K.; et al. *Bull. Mater. Sci.* **2001**, *24*, 129–135.
184. Cao, Y.; Shibata, S.; Fukumoto, I. *Composites, Part A* **2006**, *37*, 423–429.
185. Sgriccia, N.; Hawley, M. C.; Misra, M. *Composites, Part A* **2008**, *39* (10), 1632–1637.
186. Pettersen, R. C. In *The Chemistry of Solid Wood*; Rowell, R. M., Ed.; Advances in Chemistry Series 207; American Chemical Society: Washington, DC, 1984; pp 984.
187. Ishiaku, U. S.; Yang, X. Y.; Leong, Y. W.; et al. *J. Biobased Mater. Bioenergy* **2007**, *1* (1), 78–86.
188. Hai, N. M.; Kim, B. S.; Lee, S. *Adv. Compos. Mater.* **2009**, *18* (3), 197–208.
189. Athijayamani, A.; Thiruchitrambalam, M.; Natarajan, U.; et al. *Polym. Compos.* **2010**, *31* (4), 723–731.
190. Brigida, A. I. S.; Calado, V. M. A.; Goncalves, L. R. B.; et al. *Carbohydr. Polym.* **2010**, *79* (4), 832–838.
191. Nazareth, S.; Mavinkurve, S. *Int. Biodeterior. Biodegrad.* **1987**, *23*, 343–355.
192. Akin, D. E.; Foulk, J. A.; Dodd, R. B.; et al. *J. Biotechnol.* **2001**, *89*, 193–203.
193. Sharma, H. S. S. *Int. Biodeterior. Biodegrad.* **1987**, *23*, 181–186.

194. Zhang, J.; Henriksson, G.; Johansson, J. *Biotechnol.* **2000**, *81*, 85–89.
195. Gulati, D.; Sain, M. *J. Polym. Environ.* **2006**, *14* (4), 347–352.
196. Ouajai, S.; Shanks, R. A. *Macromol. Mater. Eng.* **2009**, *294* (3), 213–221.
197. Bledzki, A. K.; Mamun, A. A.; Jaszkiewicz, A.; et al. *Compos. Sci. Technol.* **2010**, *70* (5), 854–860.
198. van de Weyenberg, I.; Ivens, J.; De Coster, A.; et al. *Compos. Sci. Technol.* **2003**, *63* (9), 1241–1246.
199. Rowell, R. M, Simonson, R. A.; Tillman, A. M. European Patent 0213,252, 1991.
200. Rowell, R. M. In *Science and Technology of Polymers and Advanced Materials*; Prasad, P. N.; Mark, J. E.; Kendil, S. H.; Kafafi, Z. H., Eds.; Plenum Press: New York, 1998; pp 717.
201. Hill, A. S. C.; Abdul Khalil, H. P. S.; Hale, M. D. *Ind. Crops. Prod.* **1998**, *8* (1), 53–63.
202. Naik, J. B.; Mishra, S. *J. Appl. Polym. Sci.* **2007**, *106* (4), 2571–2574.
203. Zhang, Y. C.; Xue, Y. B.; Toghiani, H.; et al. *Compos. Interface* **2009**, *16* (7-9), 671–686.
204. Botaro, V. R.; Siqueira, G.; Megiatto, Jr., J. D.; et al. *J. Appl. Polym. Sci.* **2010**, *115*, 269–276.
205. Olfat, Y. M.; Ahmed, N.; Waleed, K. E. *J. Appl. Polym. Sci.* **1994**, *54*, 519–524.
206. Matsuda, H. In *Chemical Modification of Lignocellulosic Materials*; Hon, D., Ed.; Marcel Dekker: New York, 1996; pp 159.
207. Wang, B.; Tabil, L.; Panigrahi, S. *Sci. Eng. Compos. Mater.* **2008**, *15* (1), 43–57.
208. Joseph, S.; Sreekumar, P. A.; Kenny, J. M.; et al. *Polym. Eng. Sci.* **2010**, *50* (9), 1853–1863.
209. Paul, A.; Joseph, K.; Thomas, S. *Compos. Sci. Technol.* **1997**, *57* (1), 67–79.
210. Jia, X. L.; Yu, Y. H.; Li, G.; et al. *J. Appl. Polym. Sci.* **2010**, *118* (2), 1060–1067.
211. Lu, J. Z.; Wu, Q. L.; McNaabb, H. S.; et al. *Wood Fiber Sci.* **2000**, *32* (1), 88–104.
212. Belgacem, M. N.; Gandini, A. *Compos. Interface* **2005**, *12* (1), 41–75.
213. Kazayawoko, M.; Balatinecz, J. J.; Woodhams, R. T. *J. Appl. Polym. Sci.* **1997**, *66*, 1163–1173.
214. Bledzki, A. K.; Reihmane, S.; Gassan, J. *J. Appl. Polym. Sci.* **1996**, *59*, 1329–1336.
215. Gassan, J.; Bledzki, A. K *Composites, Part A* **1997**, *28*, 1001–1005.
216. Felix, J. M.; Gatenholm, P. *Appl. Polym. Sci.* **1991**, *42*, 609–620.
217. Keener, T. J.; Stuart, R. K.; Brown, T. K. *Composites, Part A* **2004**, *35* (3), 357–362.
218. Panthapulakkal, S.; Sain, M.; Law, S. *Polym. Int.* **2005**, *54* (1), 137–142.
219. Woodhams, R. T.; Thomas, G.; Rodgers, D. K. *Polym. Eng. Sci.* **1984**, *24* (15), 1166–1171.
220. Krzysik, A. M.; Youngquist, J. A.; Myers, G. E. *Int. J. Adhes. Adhes.* **1991**, *11* (4), 235–240.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch013

221. Kim, S. W.; Lee, S. H.; Kang, J. S. *Int. J. Thermophys.* **2006**, *27* (6), 1873–1881.
222. Elsabbagh, A.; Steuernagel, L.; Ziegmann, G. *J. Appl. Polym. Sci.* **2009**, *111* (5), 2279–2289.
223. Osman, H.; Ismail, H.; Mustapha, M. *J. Compos. Mater.* **2010**, *44* (12), 1477–1491.
224. Schneider, M. A.; Brebner, K. I. *Wood Sci. Technol.* **1985**, *19*, 67–73.
225. Colom, X.; Carrasco, F.; Pages, P.; et al. *Compos. Sci. Technol.* **2003**, *63* (2), 161–169.
226. Torres, F. G.; Cubillas, M. L. *Polym. Test.* **2005**, *24* (6), 694–698.
227. Abdelmouleh, M.; Boufi, S.; Belgacem, M. N.; et al. *Compos. Sci. Technol.* **2007**, *67* (7-8), 1627–1639.
228. Maldas, D.; Kokta, B. V. *J. Adhes. Sci. Technol.* **1989**, *3*, 529–539.
229. George, J.; Sreekala, M. S.; Thomas, S. *Poly. Eng. Sci.* **2001**, *41* (9), 1471–1485.
230. Sreekala, M. S.; Thomas, S. *Compos. Sci. Technol.* **2003**, *63* (6), 861–869.
231. Karmarkar, A.; Chauhan, S. S.; Modak, J. M.; et al. *Composites, Part A* **2007**, *38* (2), 227–233.
232. Ashori, A.; Nourbakhsh, A. *J. Appl. Polym. Sci.* **2009**, *111* (4), 1684–1689.
233. Zhang, M. Q.; Rong, M. Z.; Lu, X. *Compos. Sci. Technol.* **2005**, *65* (15−16), 2514–2525.
234. Finkenstadt, V. L.; Liu, C. K.; Cooke, P. H.; et al. *J. Polym. Environ.* **2008**, *16*, 19–26.
235. Narayan, R.; Balakrishnan S.; Shin, B. Y. U.S. Patent 7,553,919, 2009.
236. Lopattananon, N.; Songkaew, S.; Thongruang, W.; et al. *Int. Polym. Process.* **2009**, *24* (3), 272–279.
237. Gardner, D. J., West, C. H.; Han, Y. S. U.S Patent 7,659,330, 2010.
238. Oliveira, F. B.; Gardrat, C.; Enjalbal, C.; et al. *J. Appl. Polym. Sci.* **2008**, *109* (4), 2291–2303.
239. Megiatto, J. D., Jr.; Silva, C. G.; Rosa, D. S.; et al. *Polym. Degrad. Stab.* **2008**, *93* (6), 1109–1121.
240. Ramires, E. C.; Megiatto, J. D., Jr.; Gardrat, C. *Biotechnol. Bioeng.* **2010**, *107* (4), 612–621.
241. *Bioplastics Magazine*; 2010, Vol. 5 Issue 1, p 8, ISSN 1862-5258.
242. Karus, M.; Kaup, M. *J. Indus. Hemp* **2002**, *7* (1), 119–131.
243. Bledzki, A. K.; Faruk, O.; Sperber, V. E. *Macromol. Mater. Eng.* **2006**, *291*, 449–457.
244. Holbery, J.; Houston, D. *JOM* **2006**, *58*, 80–86.
245. Drzal, L. T. Mehta, G. Misra, M. Patent Appl. PCT/US2004/034202, 2004.
246. Benyus, J. M. *Biomimicry: Innovation Inspired by Nature*; Harper Collins Publishers, Inc.: New York, 2002, ISBN 9780060533229.
247. Heinzeller, T.; Nebelsick, J., Eds.; *Echinoderms*; Taylor & Francis: London, 2004.
248. Capadona, J. R.; Shanmuganathan, K.; Tyler, D. J.; et al. *Science* **2008**, *318*, 1370–1374.
249. Trotter, J. A.; Tipper, J.; Lyons-Levy, G.; et. al. *Colloquium on Biotechnology of Extracellular Matrix*; Portland Press: Leeds, 2000; pp 357.

250. McKittrick, J.; Chen, P. Y.; Tombolato, L.; et al. *Mater. Sci. Eng. C.* **2010**, *30*, 331–342.

251. Delhom, C. D.; White-Ghoorahoo, L. A.; Pang, S. S. *Composites, Part B* **2010**, *41*, 475–481.

252. Heijungs, R.; Guinee, J. B. *Environ. Manage.* **1995**, *19* (5), 665–668.

253. Wötzel, K.; Wirth, R.; Flake, R. *Angew. Makromol. Chem.* **1999**, *272*, 121–127.

254. Patel, M.; Bastioli, C.; Marini, L.; et. al. *Encyclopedia Biopolymers*; Wiley-VHC: 2003; Vol. 10, pp 409−452.

255. Joshi, S. V.; Drzal, L. T.; Mohanty, A. K.; et al. *Composites, Part A* **2004**, *35*, 371–376.

256. Corbiere-Nicollier, T.; Laban, B. G.; Lundquist, L.; et al. *Resour. Conserv. Recy.* **2001**, *33*, 267–287.

257. EPEA, Germany. http://epea-hamburg.org/epea-gmbh.html.

258. Diener, J.; Siehler, U.; et al. *Angew. Makromol. Chem.* **1999**, *272*, 1–4.

259. Ladhari, A.; Daly, H. B.; Belhadjsalah, H.; et al. *Polym. Degra. Stab.* **2010**, *95*, 429–439.

260. Marques, P. A. A. P.; Trindade, T.; Neto, C. P. *Compos. Sci. Technol.* **2006**, *66*, 1038–1044.

261. Gill, R. S.; Marques, M.; Larsen, G. *Microporous Mesoporous Mater.* **2005**, *85*, 129–135.

262. Sequeira, S.; Evtuguin, D. V.; Portugal, I. *Mater. Sci. Eng., C* **2007**, *27*, 172–179.

263. Sequeira, S.; Evtuguin, D. V.; Portugal, I. *Polym. Compos.* **2009**, *30*, 1275–1282.

264. Fong, H.; Liu, W.; Wang, C. S.; et al. *Polymer* **2002**, *43*, 775–780.

265. Son, W. K.; Youk, J. H.; Lee, T. S.; et al. *Macromol. Rapid. Commun.* **2004**, *25*, 1632–1637.

266. Wutticharoenmongkol, P.; Sanchavanakit, N.; Pavasant, P.; et al. *Macromol. Biosci.* **2006**, *6*, 70–77.

267. Junkasem, J.; Rujiravanit, R.; Grady, B. P.; et al. *Polym. Int.* **2010**, *59* (1), 85–91.

268. Hubbe, M. A.; Rojas, O. J.; Lucia, L. A.; et al. *BioRes.* **2008**, *3* (3), 929–980.

269. Zhang, K.; Ma, Y.; Francis, L. F. *J. Biomed. Mater. Res.* **2002**, *61* (4), 551–563.

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Chapter 14

Development of Biodegradable Polymer Composites

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Poly(3-hydroxybutyrate) (PHB) is a biodegradable polyester produced directly through biological processes by bacteria. Production of pure PHB requires extraction of PHB from bacterial cells and subsequent purification, both of which have substantial environmental impacts. Direct processing of PHB-laden cells as a component in a polymer composite is a solution to this issue. This research developed a wood plastic composite (WPC) comprising PHB, bacterial cell debris (cell mass), and wood fiber using an extrusion-injection molding two-step process and a direct extrusion process. Processing condition study showed that the ternary composite could be produced using common plastic processing methods. Mechanical and water resistance tests demonstrated that optimized formulations of the composite possessed properties comparable to or even better than those of a commercial WPC. The contribution of the cell mass to composite properties was identified and its mechanisms were determined. Interfacial bonding between the wood fiber and PHB was found to be important to mechanical properties and water resistance of the composite and it could be effectively improved by a coupling agent. This research demonstrated technical and economic viability of industrial production of the PHB based WPC by directly extruding PHB-laden bacterial cells with wood fibers.

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

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Introduction

A biopolymer is a polymeric substance formed in a biological system. Throughout human history, many biopolymers have been derived from biological materials (i.e. biomass) and utilized in a wide range of applications. Examples of important biopolymers include natural rubber, cellulosic polymers, protein, starch, lignin, and rosin. To increase the utilization and production of biopolymers, new processing and modification methods for biomass have been continuously developed. New monomers and replacement monomers for petrochemical products based on biomass have also been developed.

Polyhydroxyalkanoates (PHAs) are a class of biodegradable polyesters produced directly through biological processes by bacteria. They are bio-synthesized inside bacterial cell bodies as carbon and energy storage when there is a deficiency condition (e.g. lack of nitrogen or oxygen) and an oversupply of carbon source. More than 300 different types of PHA-producing microorganisms have been found to date. High productivity of PHAs (e.g. higher than 80% dry cell weight) has been reported on several of them such as *Ralstonia eutropha*, *Alcaligenes latus*, and *Azotobacter vinelandii* (*1*). PHAs can also be produced in plant leaves (e.g. switchgrass) when the plant is genetically modified to express the bacterial genes which are responsible for the biosynthesis of the polymers (*2*). PHAs can have different side groups and different numbers of carbon atoms in the repeating units, forming a family of polyesters. Due to their structural variations, the properties of PHAs cover a large envelope, ranging from brittle to ductile mechanical behaviors. For example, poly(3-hydroxybutyrate) (PHB) homopolymer is brittle and highly crystalline with a T_m around 175 °C and possesses a tensile strength comparable to that of polypropylene (PP). Its copolymer with hydroxyvalerate (HV), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), has lower crystallinity, crystallization rate, T_g, T_m, and tensile strength due to the disruption of the regular structure of PHB (*1*). The copolymer becomes more ductile with increasing HV content. Bacterial genes and feeding conditions can be modified to regulate the molecular structures of the produced PHAs. Production rate of PHAs depends on bacterial strains and the living environments of the bacteria such as pH value, oxygen level, carbon and nitrogen sources, etc.

PHAs can be harvested from bacterial cells through an extraction and purification process after a preset polymer production rate is reached. This process is a key step in producing PHAs because it significantly influences the purity, properties, and production costs of the polymer. Many extraction methods have been developed including solvent extraction, digestion, mechanical disruption, supercritical fluid, air classification, etc (*3–7*). To facilitate polymer extraction, pretreatment steps such as heat pretreatment, salt pretreatment, alkaline pretreatment and freezing are often used to accelerate cell disruption (*8*, *9*). The obtained polymer from the extraction process is purified by hydrogen peroxide treatment in combination with enzymes or by ozone treatment. The bacterial cell residual after PHA extraction is termed cell mass. The cell mass comprises mainly polysaccharides, protein, and lipid.

The extraction and purification process of PHAs is an energy and chemical agent intensive process. It significantly increases the production cost of PHAs and reduces the environmental and energy benefits associated with the polymer. On the other hand, the components of the cell mass such as polysaccharides and protein have been successfully utilized to prepare polymer composites using ordinary polymer melt processing techniques (*10–12*). Therefore it is possible to directly process PHAs and their accommodating bacterial cells together without prior separation. This would bring significant energy and environmental benefits by eliminating the costly extraction and purification steps.

One suitable application for the direct processing of PHAs and cell mass would be on wood plastic composites (WPCs), which are produced by blending a molten polymer with wood fiber (WF). WF is attractive in developing polymer-cellulosic fiber composites because of its low cost and high specific properties. WPCs have many properties superior to those of traditional wood products, such as moisture resistance, fungus and termite resistance, dimensional stability, and ease of maintenance (*13*, *14*). WPCs are a rapidly growing product area, averaging a 38% growth rate since 1997. WPCs are traditionally dependent on petroleum based thermoplastics, i.e. polyethylene(PE), polypropylene(PP), polystyrene(PS) etc., increasing their overall energy costs by over 230% when compared to traditional engineered wood products (EWP). PHAs are not currently used in WPCs primarily because their production costs are about 2 ~ 3 times higher than those of conventional petrochemical-derived plastics. However, if considering the costs and procedures of recycling the petroleum based thermoplastic, the overall life-cycle costs of PHAs will be much lower. The production costs of PHA containing WPCs can be further reduced by directly extruding PHAs and their accommodating cells together with WF. The PHA-laden cells from bioreactor are dried and used directly to replace petroleum-derived plastics to make WPCs. Moreover, the bacteria can be grown in waste effluents from the municipal, agricultural, and forest products sectors. Using this strategy, the cost of utilizing PHAs in WPCs can be greatly reduced.

It is estimated that annual energy savings of over 42 trillion BTU by 2020 can be achieved by using the proposed technology. If it deploys across the building/ construction industry, the potential for 310 trillion BTU can be saved annually (*15*). Significant environmental benefits will also be realized by the wastewater treatment industry in the municipal, industrial (pulp mill), and agricultural sectors through incorporation of waste biosolids into composites. Improved economic competitiveness of the domestic forest products industry is expected, as the plastic in WPCs comprises on average 52% of formulation costs and 30% of total product costs.

Coats utilized PHB-rich cell mass to produce wood fiber reinforced PHB composites (*16*). They demonstrated that the PHB-rich cell mass could be directly used to prepare the composites by melt blending and pressure molding, therefore eliminating the costly PHB separation and purification processes. However, this study lacks the investigation into the microstructure and interface of the composites, which often critically determines composite properties. The impact of cell mass content on mechanical properties and water resistance of the composites and the reasons behind the impact are also not fully identified. Water

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resistance is an important property for WPCs due to the environments which most WPCs are exposed to in applications. In addition, the composites in this study were developed using a torque rheometer (for blending) and a capillary (for pressure molding), which were not representative industrial processing methods.

In this research, a WPC comprising PHB, cell mass, and wood fiber (WF) was developed using common industrial processing methods. The processability and properties of the WPC was investigated and compared with a commercial WPC. A PHB/WF binary composite was first developed and its processing conditions, properties, and especially interfacial compatibilization techniques were studied. Based on the obtained results, different percentage of cell mass was introduced to the binary composite and its influence to the properties of the composites was studied. Finally a PHB/WF/Cell mass ternary WPC was extruded using the conditions similar to those found in industrial productions. Mechanical properties and water resistance of this extruded WPC were compared with the commercial WPC. This study paved the way for industrial production of the PHB based WPC by directly extruding PHB-laden bacterial cells with wood fibers.

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Experimental Section

Materials

The PHB/WF composites were primarily composed of PHB (Tianan Biologic Material Co., Ltd., Ningbo, China) and 60-mesh ponderosa pine wood fiber (American Wood Fibers, Schofield, WI). Boron nitride (BN) (Carbotherm PCTF5, Saint Gobain Advanced Ceramics Co., Amherst, NY) was used as a nucleation agent to promote PHB crystallization (Qian 2007). Glycolube WP2200 (Lonza Inc., Allendale, NJ) was included as a lubricant to improve composite processability. Talc (Nicron 403 from RioTinto of Centennial, CO.) was used to improve processing and water resistance of the highly filled composites. Liquid polymeric methylene diphenyl diisocyanates (pMDI, Mondur 541 from Bayer MaterialScience, Pittsburgh, PA) contained 31.5 mass% NCO functional group and was used as a coupling agent between the hydrophobic PHB and the hydrophilic WF and cell mass. The cell mass used in this research was provided by University of California – Davis. The bacteria were grown in municipal waste effluents until PHA harvest. The cell mass was dried and ground into 60-mesh particles using a hammer mill.

PHB/WF Composite Preparation

60-mesh pine wood fiber was dried in a rotary steam tube drier to 3% moisture content before use. Ground cell mass particles were dried at 100 °C for 24 hours in a convection oven. PHB, BN, WP2200, and talc were used as received. All the composite samples were prepared by extrusion blending followed by injection molding. More specifically, 35 parts PHB, 57 parts WF, 8 parts Talc, 0.2 parts BN, and 3 parts WP2200 were first manually mixed in a Ziploc plastic bag by vigorous shaking and tumbling. For the formulations containing liquid pMDI, the liquid was added to the PHB powder and dispersed using a standard kitchen blender for

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1 minute before the PHB powder was mixed with the other components in the plastic bag. The mixtures were then compounded using a co-rotating twin screw extruder (Leistriz ZSE-18) fed by a volume feeder. The screw measured 18 mm in diameter with an L/D ratio of 40. To improve melt strength and reduce thermal degradation, a declining temperature profile of the extruder was adopted for the compounding. From the feeding throat to the die, the temperatures were set at 170, 175, 170, 165, 164, 163, 162, and 160 °C. The screw speed was maintained at 125 rpm. Under this speed, the residence time of the materials in the barrel was estimated to be about 1.5 minutes. After exiting a strand die, the extrudate was air cooled and pelletized for injection molding.

Flexural test specimens (12 × 3 × 127 mm) and tensile specimens (ASTM standard D638 type I) were injection molded (Sumitomo SE 50D). A temperature profile of 175, 180, 175, and 170 °C from the feeding zone to the nozzle was used for the injection molding. Mold temperature was 60 °C. The mold filling pressure was set at 1700 kgf/cm^2 and the packing pressures were 1250 kgf/cm^2 and 1360 kgf/cm^2 for the 1st and 2nd stage packing, respectively. The filling time was set at 8 seconds. The first and the second stage packing time were 1 and 2 seconds, respectively. The cooling time was set at 40 seconds. The total cycle time was about 50 seconds.

Impact test specimens (12 × 3 × 60 mm) were prepared following ASTM D256. All the specimens were notched by a XQZ-I specimen Notch Cutter. The specimens for water absorption test were cut by a milling machine to the size of 11 × 2.5 × 125 mm.

Testing

A screw driven Instron 4466 equipped with a 10-KN load cell was used for tensile and flexural tests. Sample strain was measured by an extensometer (MTS model # 634.12E-24). Crosshead speed of 5 mm/min was used. Impact tests were conducted using a Dynisco Basic Pendulum Impact tester. ASTM standard D638, D790, and D256 (mode A) were followed in the tensile, flexural, and impact tests, respectively. Five replicates were tested for each formulation to obtain a mean value. Sample density was calculated by dividing sample mass by sample volume. All the samples (for both mechanical and density testing) were conditioned at 23 °C and 50% relative humidity (RH) for 7 days prior to the tests.

Dynamic mechanical properties of the modified systems were analyzed with a Rheometric Scientific RSA II using three-point-bending configuration using a temperature ramp test mode. The linear viscoelastic range of the composites was determined beforehand through a strain sweep test. The temperature ramp tests were conducted from -30 °C to 125 °C using a constant frequency of 1 Hz and a strain amplitude of 0.03% (within the linear viscoelastic range).

Water absorption of the composites was performed following ASTM D570. Samples were immersed in distilled water at room temperature. The weight and thickness of the samples were measured after different periods of immersion time. Moisture content (MC) and thickness swelling (TS) were calculated by the following equations:

$$MC(\%) = \frac{M - M_0}{M_0} \times 100$$

$$TS(\%) = \frac{T - T_0}{T_0} \times 100$$

Where M and T are the mass and thickness of the specimen at time t, and M_0 and T_0 are the initial (dry) weight and thickness, respectively.

To investigate wood fiber and cell mass distribution in the PHB matrix and particle-polymer interfacial bonding, tensile fracture surfaces of the composites were sputter coated with gold and their morphology were studied using a Hitachi S-570 scanning electronic microscope (SEM). The composites were also sliced with a microtome to obtain flat cross sectional areas. The areas were investigated using SEM for more microstructural information such as wood cell structure, particle-polymer interface, and polymer melt infusion.

Results and Discussion

Properties of PHB/WF Composites

The influence of WF and pMDI on the mechanical properties of the composites is demonstrated in Figure 1. PHB without WF (still comprising other additives: talc, BN, WP2200, and 4% pMDI) shows a tensile strength and modulus of 32 MPa and 4.7GPa, respectively. The addition of WF significantly increased the modulus to 8.4GPa but decreased the strength to 22.5 MPa. These property changes are common to many fiber reinforced polymer composites where interfacial bonding between the fiber and the polymer matrix is not strong. By introducing 1, 2, and 4% of pMDI into the system as a coupling agent, the modulus and strength of the composite were both steadily increased. Especially, the composite containing 4% of pMDI shows a tensile strength even higher than that of the PHB without WF (34.6 vs 32 MPa), an indication of strong interfacial bonding between the PHB matrix and the WF particles. The addition of 4% pMDI also slightly increased failure strain of the composite from 0.37% (without pMDI) to 0.47%. The low failure strain is due to PHB’s brittle nature and high content of WF in the composite. The failure strain can be improved by using PHBV copolymer (instead of PHB homopolymer) to increase the ductility of the composite matrix. In conclusion, with 57 parts of PHB polymer being replaced by low cost wood fiber, the composite shows higher mechanical properties than does the PHB without WF. These results imply the great advantages of PHB/WF composites, i.e. low production costs and high mechanical properties compared to the neat PHB polymer.

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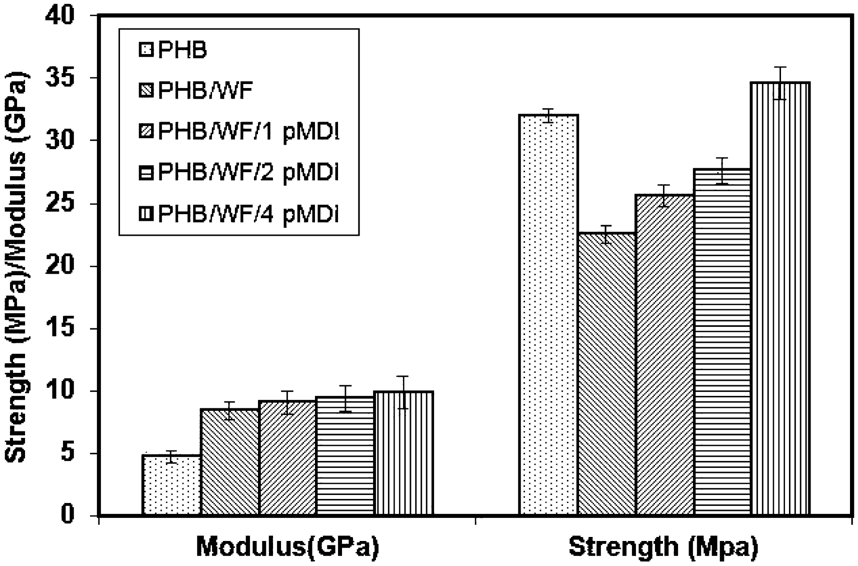

Figure 1. Comparison of tensile strength and modulus between different formulations of the PHB/WF composites.

The results from DMA testing complement those from tensile testing. As shown in Figure 2, with increasing pMDI content, elastic modulus E' of the composite increases and the loss modulus E" decreases. The damping (loss factor Tanδ) of the composite also decreases with increasing pMDI content. These trends confirm that the composite becomes stiffer after the addition of pMDI. The reason is due to increased interfacial bonding between PHB and WF, which decreases PHB molecular mobility in the vicinity of WF surfaces.

Water resistance is a critical property for WPCs. The transport of liquids and gases through a solid medium is often described by Fickian diffusion. Fick's second law describes the transport of molecules through a medium in which the diffusion flux and concentration gradient at a particular point change with time. Under conditions of non-steady state diffusion, the apparent diffusion constant, D_A may be described by:

$$D_A = \pi \left[\frac{h}{4M_{sat}}\right]^2 \left[\frac{\partial M_t}{\partial \sqrt{t}}\right]^2$$

Where h is the thickness of the sample, M_t is the moisture content at time t, M_{sat} is the moisture content at saturation, and $\partial M_t/\partial\sqrt{t}$ is the slope of the moisture uptake versus square root of time (Chowdhury and Wolcott, 2007). Water absorption plots exhibit Fickian behavior when $Mt/\sqrt{t}$ exhibits a linear relationship.

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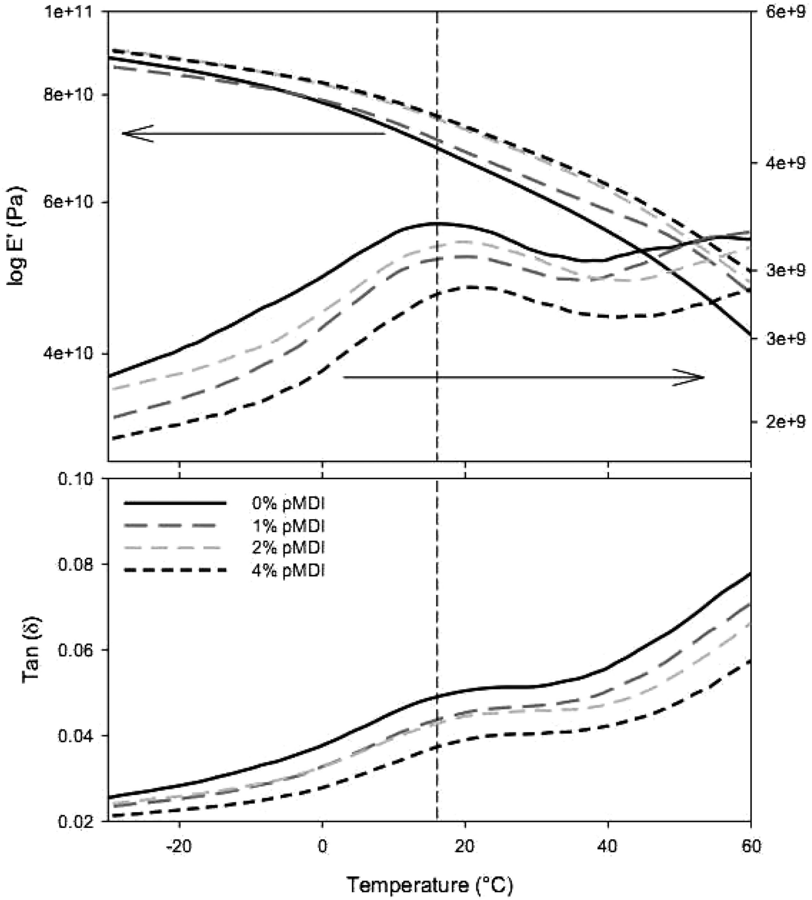

Figure 2. DMA results for PHB/WF composites containing different content of pMDI.

Figure 3 compares water absorption of the PHB/WF composites containing no and 4% pMDI coupling agent. Moisture content was plotted as a function of the square root of time in this figure. Both of the curves in Figure 3 show deviation from Fickian behavior after certain periods of time. The inflection points for PHB/WF and PHB/WF/pMDI are about 400 $s^{1/2}$ and 725 $s^{1/2}$, respectively. The inflection points were found to coincide with the onset of cracking of the composite samples (visual observation). It is believed that this sample cracking is the reason for the deviation from Fickian behavior. Roy and Xu (2001) indicated that the deviation could occur when sample defects were present or when the sample approached saturation. The cracking of the composite samples may be caused by their hygrothermal swelling of WF, which induces internal stresses within the composites. The presence of these cracks may also increase the moisture content at saturation due to the increase in surface area or facilitating capillary uptake of moisture.

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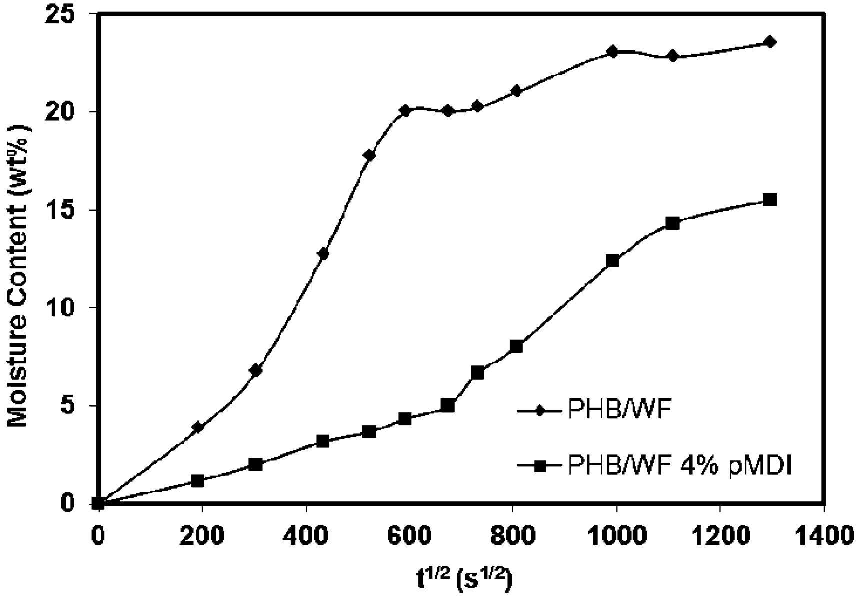

Figure 3. Comparison of water resistance of the PHB/WF composites comprising zero and 4% pMDI coupling agent.

When strong WF and PHB interfacial bonding is absent, micron scale gaps between the WF fiber and the matrix and micro-cracks at the interface may form interconnected pathways for moisture transportation. If strong interfacial bonding is generated due to the reactions of the coupling agents, most of these pathways are closed and therefore the transportation of moisture can be significantly hindered. As a result, in Figure 3 the composite containing pMDI shows much lower moisture content (at any immersion time) and lower moisture absorption rate (initial slope) compared to the composite without pMDI. The water absorption behavior was also recorded for composite modified with 1% and 2% pMDI. While not shown, the increase in pMDI content from zero to 4% reduced the water absorption rate, increased the time before cracking and the deviation from fickian behavior. Similar results were found by Zhang (2005) that the addition of pMDI reduced the water absorption rate and the moisture content at saturation of polyethylene/WF composites.

The above results demonstrate that bacterial polyester PHB is suitable for producing WPC. Without the coupling agent, tensile strength of PHB was reduced after mixing with WF. Water resistance also decreased due to the hydrophilicity of WF. However, both properties were significantly improved after the application of the coupling agent. Especially, mechanical properties of the PHB/WF containing 4% pMDI were higher than those of the neat PHB. Based on these promising results, cell mass was introduced to the PHB/WF binary system, with its content varied from low to high. The impact of cell mass to the properties of the composite was investigated so that optimized formulation could be identified for the PHB/WF/Cell mass ternary composite.

Properties of PHB/WF/Cell Mass Ternary Composite

In the ternary composites, the content of PHB and all the additives were maintained constant. Four parts of pMDI were used as the coupling agent. Part of WF (20, 40, 60, and 80%) was replaced with the same amount of cell mass to produce four different ternary composite formulations. PHB/WF (PW) and PHB/Cell mass (PC) binary composites were also prepared as the control formulations. The details of all the formulations are given in Table I. All the formulations were made into test samples and were tested using the same methods and conditions adopted for the PHB/WF binary composite.

Density is an important property parameter for WPC products. WPCs generally show higher density compared to engineered wood due to the relatively high density of WPC's polymer matrix. By changing the content of the cell mass, the density of the ternary composite may be affected. Figure 4 compares the density of the ternary composites containing different content of cell mass. It appears that all the samples have similar density values (variation within 2%). This may be due to similar density values of the wood cell wall of WF (~1.5 g/cm^3) and cell mass. The empty spaces in WF (e.g. lumens and cell cavities) lower WF density. However, during injection molding of the composites, the spaces were filled up with high pressure PHB melt (further discussion in composite morphology). Under this situation, the density of WF is essentially equal to that of the wood cell wall. The results in Figure 4 indicate that the cell wall density was close to that of the cell mass.

Table I. Formulations of PHB/WF (PW), PHB/Cell mass (PC) and PHB/WF/Cell mass (PWC) composites. All units are in parts.

Formulation/ Components	*PW*	*PWC20*	*PWC40*	*PWC60*	*PWC80*	*PC*
Cell mass	0	11.4	22.8	34.2	45.6	57
WF	57	45.6	34.2	22.8	11.4	0
PHB	35	35	35	35	35	35
Talc-Nicron 403	8	8	8	8	8	8
pMDI	4	4	4	4	4	4
WP2200	3	3	3	3	3	3
Boron Nitride	0.2	0.2	0.2	0.2	0.2	0.2

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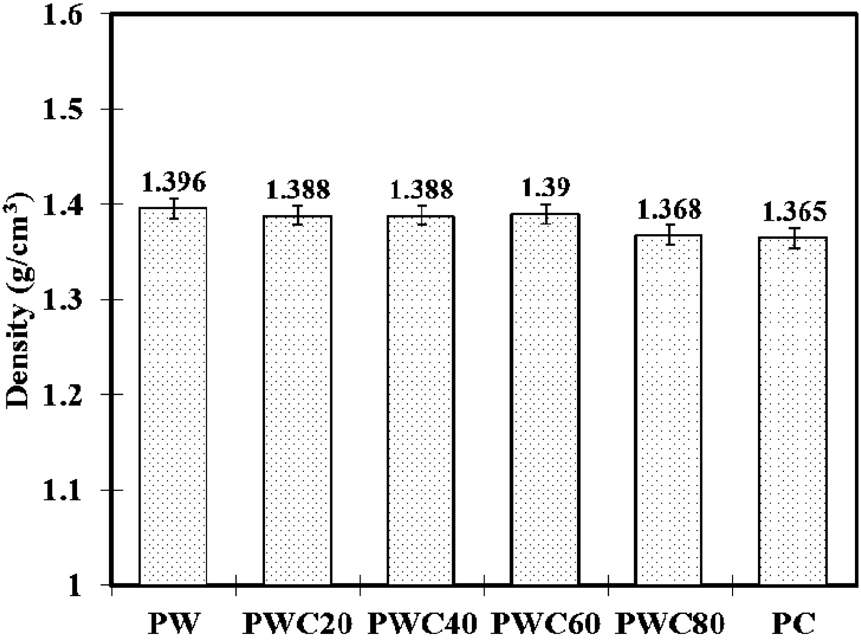

Figure 4. Comparison of density of the PHB/WF/Cell mass composites.

Figure 5 compares tensile strength and Young's modulus of the composites. Both the strength and the modulus show a declining trend with increasing cell mass content. For instance, when 60% of the WF was replaced by cell mass (PWC60), tensile strength of the ternary composite was 55% of that of the PW control. The declining rate of the modulus is lower compared to that of the strength. The modulus of PWC60 is 66% of that of the control. Similar declining trends were also observed on flexural properties and impact strength of the composites (Figures 6 and 7). These trends were believed to be due to several reasons. The interfacial bonding between cell mass and PHB was weaker than that between WF and PHB (more discussion in composite morphology). Cell mass started thermal degradation at 100 °C (based on TGA tests). The degradation of the cell mass produced gaseous substance which could cause voids and other sample defects in the composites. WF wall was expected to have higher strength and modulus than cell mass due to the lipid and non-cellulose polysaccharide ingredients contained in cell mass. Moreover, the cell mass particles were in cubic shape (see composite morphology), which was unfavorable to the mechanical properties of the composites compared to fiber-shaped WF.

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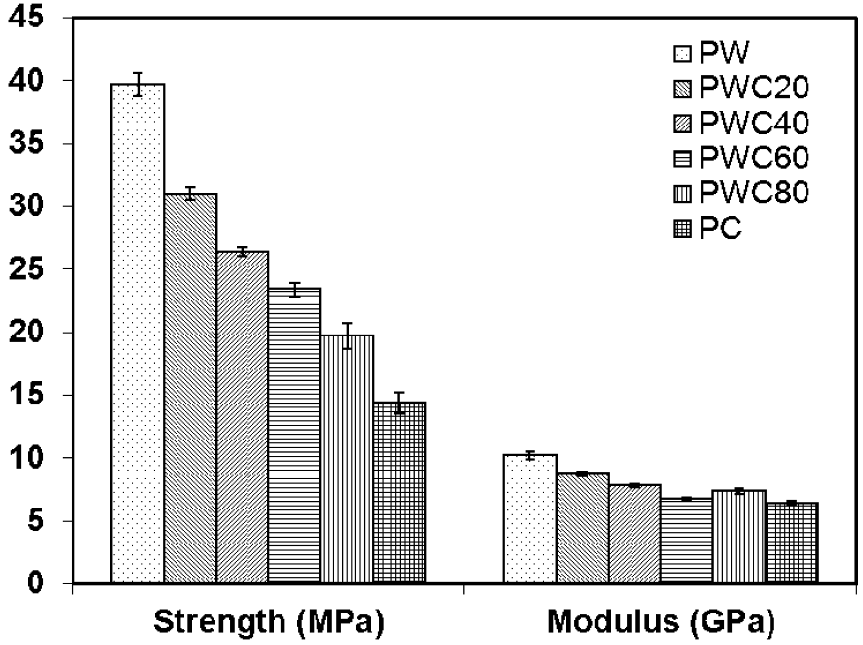

Figure 5. Tensile properties of the PHB/WF/Cell mass composites.

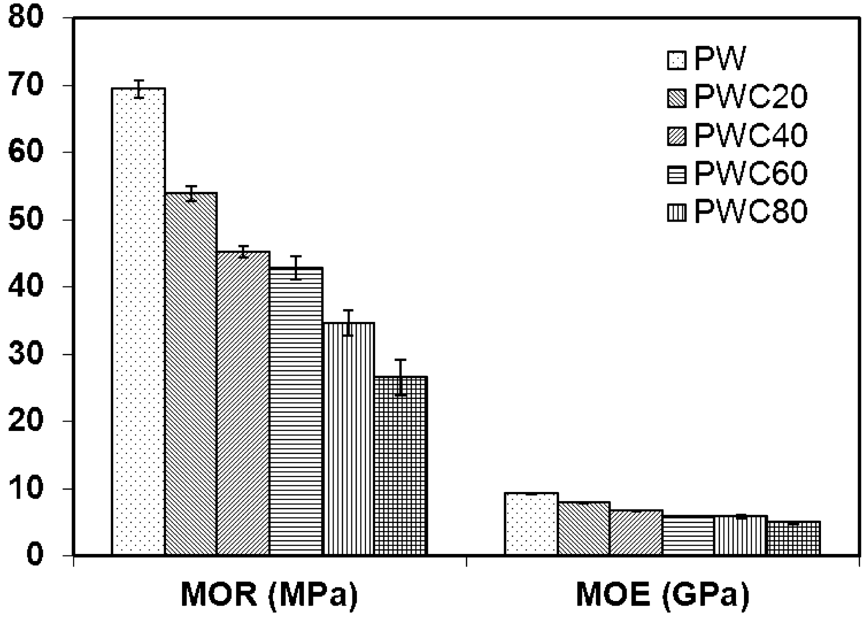

Figure 6. Flexural properties of the PHB/WF/Cell mass composites.

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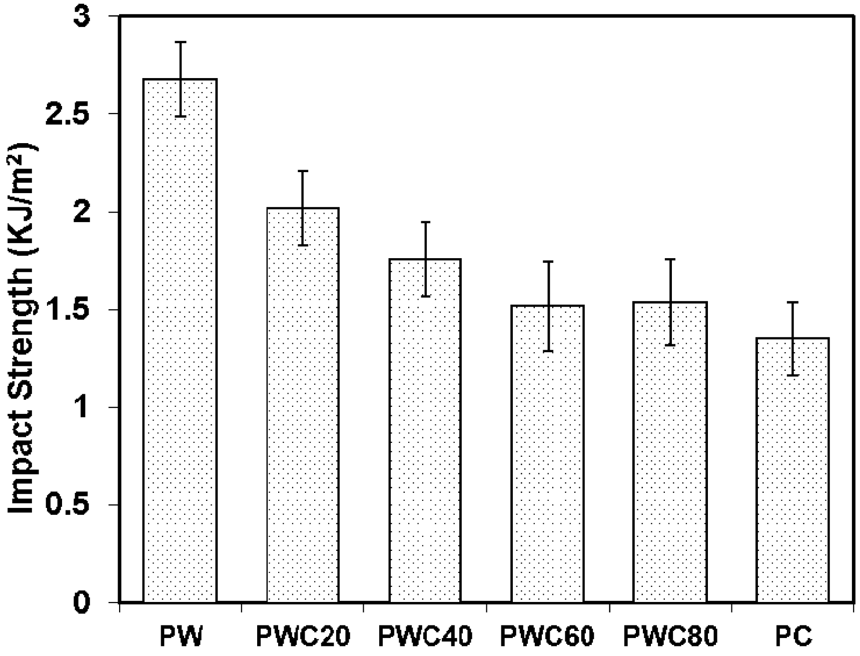

Figure 7. Impact strength of the PHB/WF/Cell mass composites.

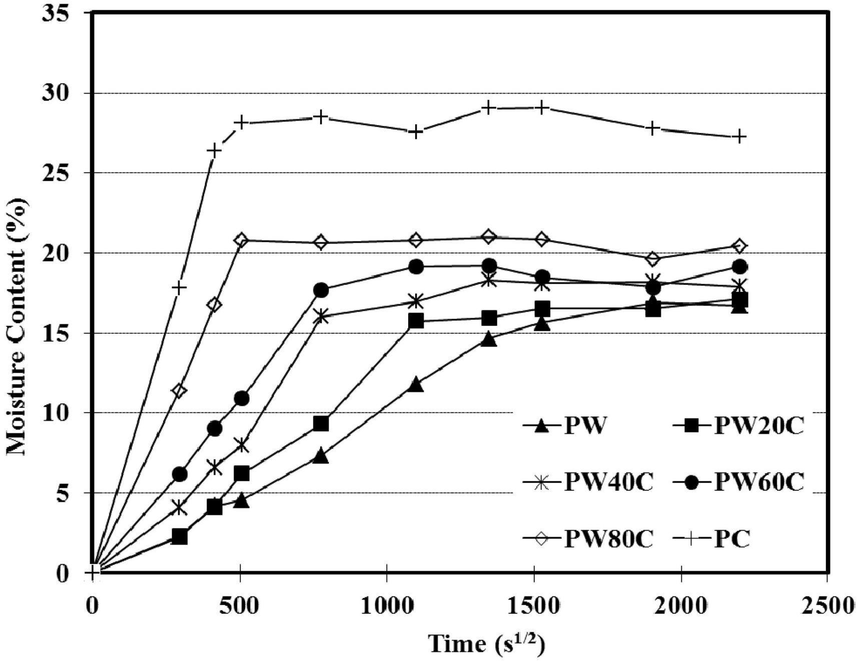

Figure 8. Water absorption of the composites during a 12-week long immersion test.

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

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Water resistance of the ternary composites was also studied to examine cell mass's impact on this property. As shown in Figure 8, moisture content increases with increasing cell mass ratio throughout a 12-week test period. The initial slope of the curves also increases, implying elevated moisture absorption rate at high cell mass content. In addition, all the curves in the figure level off (moisture saturation) after different length of time. The samples containing higher content of cell mass show shorter time to reach saturation. These results indicate that cell mass accelerates water absorption of the composites and the samples with higher cell mass content take less time to reach moisture saturation. For example, sample PC, with all its WF being replaced with cell mass, reached its moisture saturation content (M_{sat}, ca. 28%) in three days. In comparison, sample PW, with no cell mass in its formulation, reached its saturation content (ca. 17%) in six weeks.

Sample thickness of the composites was also monitored during the immersion test. The percentage of thickness increase (thickness swelling) as a function of the square root of time is plotted in Figure 9 for all the composites. Comparing this figure to Figure 8, it is obvious that the curves from both figures show similar trends, i.e. the thickness swelling increases with increasing cell mass content of the composites.

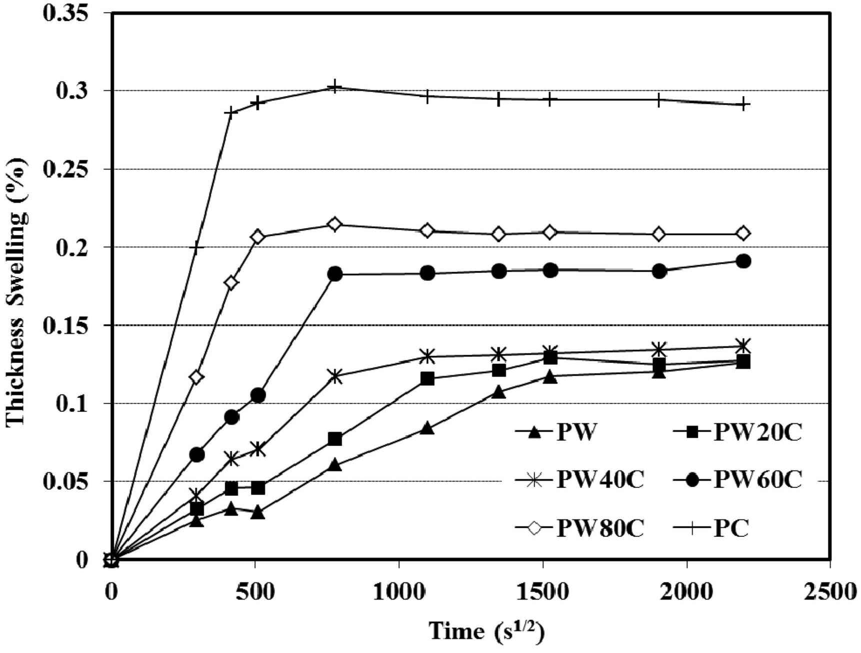

Figure 9. Thickness swelling of the composites during a 12-week long immersion test.

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Using the equation given earlier, the apparent diffusion coefficient (D_A) of the composites can be calculated from Figure 8. For 3-dimensional diffusion, geometric edge correction can be applied to calculate the true diffusion constant D (*17*):

$$D = \frac{D_A}{\left(1 + \frac{h}{L} + \frac{h}{W}\right)^2}$$

where h, L, and W are the thickness, length, and width of the specimen, respectively. The degree of sample swelling was defined by a swelling coefficient (*β*):

$$\beta = \frac{\text{Thickness swelling at saturation}}{\text{Moisture content at saturation}} = \frac{TS_{sat}}{MC_{sat}}$$

The composites swell because they absorb water during the immersion test. The more water they absorb, the higher the degree of swelling. However, the internal structure of the composites is also a factor affecting how much the samples swell. At the same moisture content, the samples with strong interfacial bonding tend to swell less compared to those with weak interfacial bonding (*18*). The swelling coefficient separates the effect of moisture absorption from interfacial bonding. High β value indicates significant contribution from poor interfacial bonding.

Table II summarizes D_A, D, β, water uptake rate, TS_{sat}, and MC_{sat} for all the composites. All the values increase with increasing cell mass content. Moisture absorption of the composites occurred at a higher rate and to a larger degree when the cell mass content increased. The increase in β implies that the weak interfacial bonding between cell mass and PHB played a significant part in the thickness swelling of the composites at high cell mass content. In conclusion, water resistance of the composites decreased after replacing different percentage of WF with cell mass. Higher cell mass content led to larger degree of water resistance deterioration. This was most probably due to high hydrophility of cell mass because composite PC had much higher moisture saturation content (28% vs. 17%) compared to composite PW. Moreover, poor interfacial bonding between cell mass and PHB allowed fast moisture penetration through their interfacial gaps.

Mechanical properties and water resistance of the PHB/WF/Cell mass composite have been shown to decrease with increasing cell mass content. The reason was found to be closely related to the microstructure of the composites. Figure 10 shows the SEM micrographs of the cell mass and WF before blending. The cell mass particles exhibit wide size distribution with the large ones measuring up to 300 microns (Figure 10a). Most of the particles show irregular shapes with a length/diameter (L/D) ratio close to one. On the other hand, WF particles are comprised of bundles of wood fibers (Figure 10b) which can be broken down into smaller fibers by the intensive shearing during extrusion. After extrusion and injection molding, the cell mass particles and WF in the composites were found to be homogenously distributed in the polymer matrix (Figure 11b and c). The cell mass particles are evident on the fracture surface (Figure 11c) due to their large size, cubic shape, and loose contact with the matrix (existence of interfacial gaps). On the other hand, WF is not easily discernable in Figure 11b because of its strong interfacial bonding with the matrix. The different level of interfacial bonding between PHB/Cell mass and PHB/WF is more obvious in high magnification micrographs (Figure 11b' and c'). In 11b' WF is shown to be tightly bound to the matrix, whereas 11c' shows that interfacial gaps exists between the cell mass particles and the matrix. The strong interfacial bonding between WF and the PHB matrix is promoted by pMDI, which is highly reactive with both hydroxyl and carboxyl groups, and is able to chemically link the two phases. Cell mass is a complicated system, comprising mainly protein, polysaccharides, triglycerides, and inorganic impurities. The pre-treatment during its production, impurities, and thermal degradation during processing may all have adverse effects on its reactivity with pMDI. As a result, cell mass and PHB cannot be compatibilized by pMDI as effectively as can WF and PHB.

Composite samples were also sectioned using a microtome to obtain a flat surface and to expose the internal structure of the cell mass particles and WF. Figures 12a and b show the sectioned surfaces of the PHB/WF/Cell mass composite at low and high magnification. Figure 12a compares the internal structure of the cell mass and WF. The cell mass particle appears solid and is surrounded by an interfacial gap, whereas the WF particle appears porous due to its cell structure and its boundary is diffusive because of its strong interfacial bonding with the matrix. At high magnification (Figure 12b and c), the cell structure of WF can be clearly seen. Many wood cells were crushed or severely deformed (indicated by arrows) due to the high pressure occurred in injection molding. The high pressure also forced PHB melt into some of the cells through longitudinal and transverse lumens (Figure 12c). The penetration of polymer into the wood cells generated a mechanical interlock between the polymer and the wood fiber, which substantially improved stress transfer between the two phases. In contrast, biomass particles seemed to be solid and could not be penetrated or deformed by PHB melt (Figure 12a). As a result, no mechanical interlocking was developed between the two phases. This is another reason for the weak interfacial bonding between the cell mass particles and the PHB matrix.

Because of the cell mass particles' large size, irregular shape and low L/D ratio, weak interfacial bonding, and thermal degradation during processing, mechanical properties and water resistance of the ternary composites were found to decrease with increasing cell mass content. These properties need to be compared with those of commercial WPCs to validate this new composite's technical and commercial viability. Current important applications for WPCs include structural members in building and construction, garden and outdoor products, interiors and internal finishes, etc. Almost all of these products are produced by extrusion. Therefore it is necessary to directly extrude the ternary composites (rather than extrusion followed by injection molding) using a condition resembling industrial production and then compare the properties of the extruded composites with a commercial WPC.

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Table II. Water absorption properties of the composites obtained from the immersion test

	PW	*PWC20*	*PWC40*	*PWC60*	*PWC80*	*PC*
D_A	6.56E-07	1.22E-06	1.96E-06	2.24E-06	6.11E-06	6.73E-06
D	4.02E-07	7.49E-07	1.19E-06	1.36E-06	3.27E-06	4.09E-06
B	0.731	0.716	0.759	0.908	0.993	1.102
Line slope*	1.08E-04	1.45E-04	2.03E-04	2.27E-04	4.06E-04	5.77E-04
TS_{sat}	12.93 %	13.66 %	15.43 %	20.12 %	20.81 %	29.41 %
MC_{sat}	17.68 %	19.09 %	20.32 %	22.16 %	20.82 %	29.05 %

* $dM_t/d\sqrt{t}$, indication of moisture absorption rate.

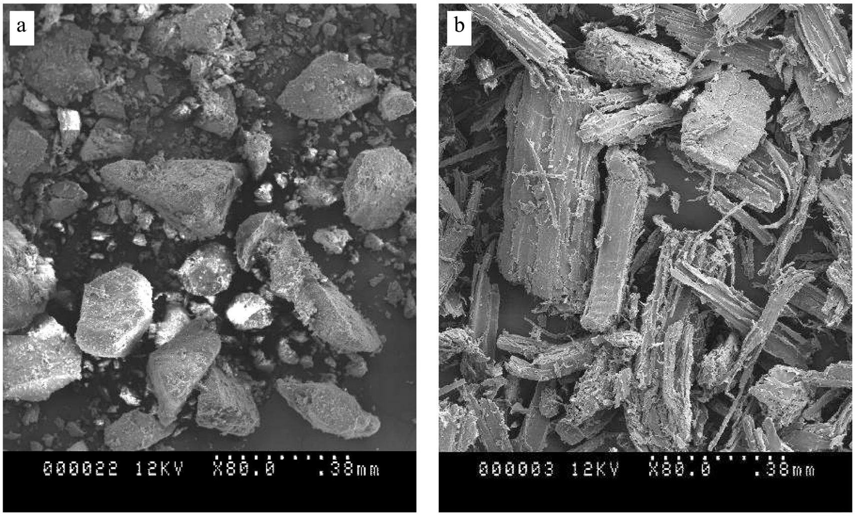

Figure 10. SEM micrographs of cell mass (a) and WF (b) particles before processing.

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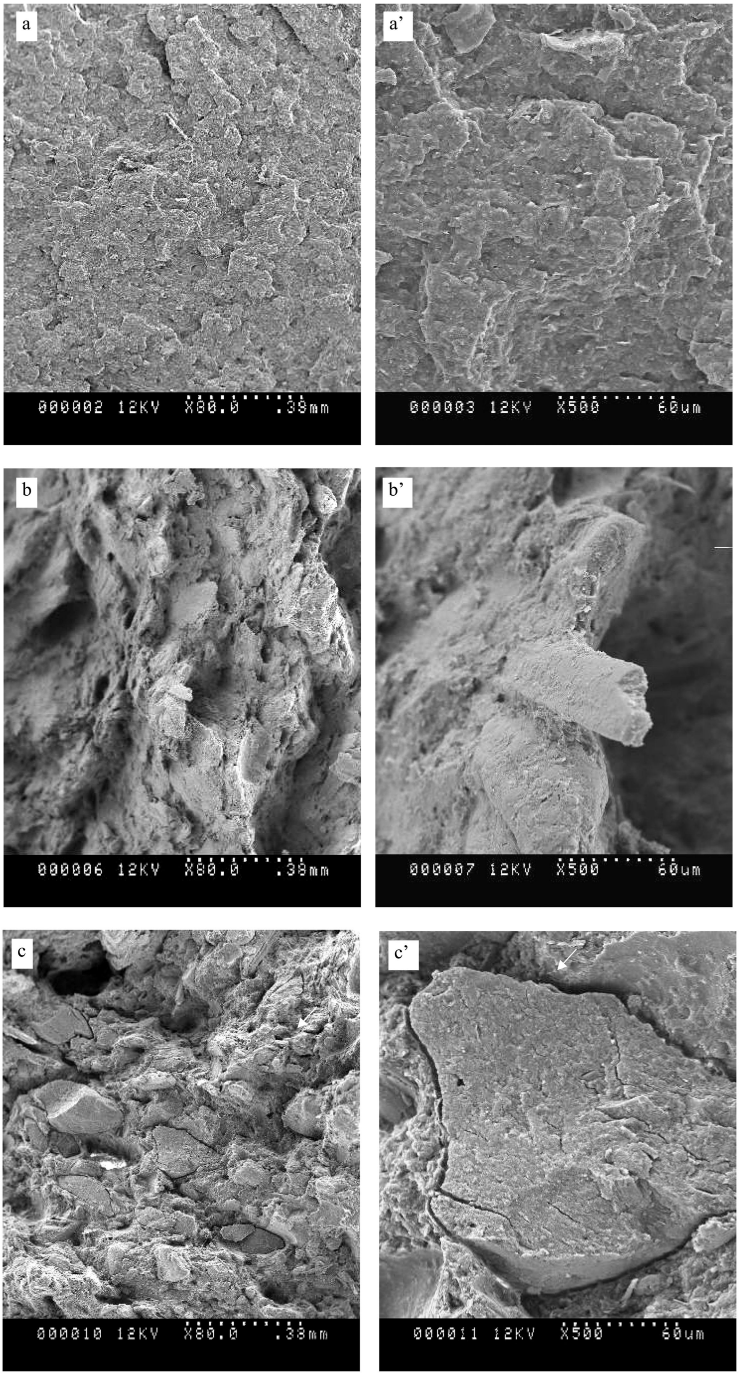
a
000002 12KV X80.0 .38mm
a'
000003 12KV X500 60um
b
000006 12KV X80.0 .38mm
b'
000007 12KV X500 60um
c
000010 12KV X80.0 .38mm
c'
000011 12KV X500 60um

Figure 11. Fracture surfaces of neat PHB (a, a'), PHB/WF composite (b, b'), and PHB/WF/Cell mass composite (c, c'). Magnification: X500.

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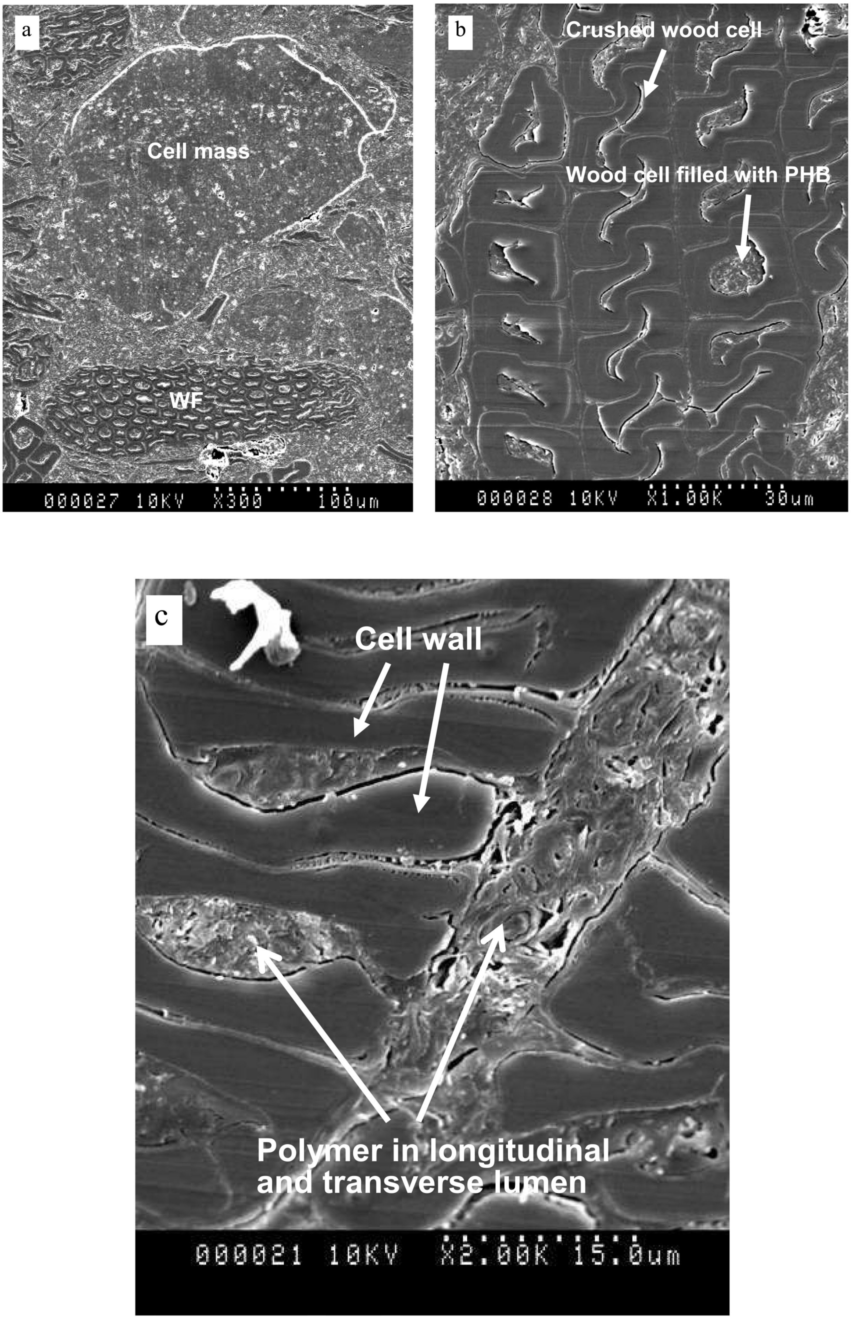
a
Cell mass
WF
000027 10KV X300 100um
b
Crushed wood cell
Wood cell filled with PHB
000028 10KV X1.00K 30um
c
Cell wall
Polymer in longitudinal and transverse lumen
000021 10KV X2.00K 15.0um

Figure 12. Sectioned surfaces of PHB/WF/Cell mass ternary composites showing that PHB melt was forced into wood cells through the lumens.

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Industrial Development of PHBV/WF/Cell Mass WPC

Four ternary composite formulations (i.e. PWC 20, 40, 60, and 80) and the two binary controls (i.e. PW and PC) were extruded throughout a slit die (38 × 9.8 mm) using a 35mm conical twin screw extruder (Cincinnati Milicron CM35). The melt strength of the materials and the die pressure was too low (especially when cell mass content was high) to produce defect-free samples if previously established extrusion temperature profile was used. Therefore, lower temperature profiles were selected for the formulations comprising high contents of cell mass. The temperature profile was determined for each formulation using a torque rheometer so that all the formulations showed similar mixing torque (and therefore viscosity) at their selected temperatures. This process ensured that each formulation experienced similar packing pressure during extrusion. All the determined temperature profiles were shown in Table III. The screw speed was maintained at 20 rpm for all the formulations. Extrusion residence time was about 1 minute. Melt pressure at the die entrance was monitored to confirm pressure similarity across the formulations. The extrudates were cooled by spray water upon exiting the die. To compared the properties of the PHB/WF/Cell mass composites with those of commercial WPCs, a WPC comprising high density polyethylene (HDPE, 32 parts), WF (58 parts), zinc stearate (2 parts), and ethylene *bis* straramide (EBS) wax (1 part), which resembles a popular commercial WPC, was also extruded (formulation code HW).

Table III. Extrusion temperature profiles used for the seven formulations

Zone/ Sample	*BZ1*	*BZ2*	*BZ3*	*Screw*	*DZ1*	*DZ2*
PW	170	175	165	163	163	160
PWC 20	170	175	165	163	163	160
PWC 40	170	170	165	160	160	160
PWC 60	165	170	165	155	155	155
PWC 80	160	165	160	150	150	150
PC	160	160	155	150	150	150
HW	163	163	163	163	170	170

BZ: barrel zone; DZ: die zone; HW: HDPE/WF

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The extruded composite bars were tested for flexural properties. Figures 13a and b compare modulus of rupture (MOR) and modulus of elasticity (MOE), respectively, of all the PHB composites and the commercial HDPE composites. First the extruded PHB composites show the same trend as the injection molded composites, i.e. decrease in MOR and MOE with increasing cell mass content. Second the MOR and MOE of the extruded composites are lower than those of the injection molded samples because the latter process provides much higher packing pressure to the samples. As a result, the samples produced by injection molding tend to have fewer defects and higher degree of polymer penetration into the wood cells. Third the figures show that the ternary composites PWC60 and PWC80 exhibit a MOR and MOE comparable to that of the commercial HW composite. The composites comprising lower cell mass content (e.g. PWC20 and 40) shows a MOR and MOE even higher than that of the commercial one.

Water resistance of the extruded samples was also tested using the same immersion method and their results are compared in Figure 14. The commercial formulation HW shows the highest moisture saturation content (MC_{sat}= ca. 22%) after ca. 1000 hrs. Its moisture absorption rate (initial line slope) is comparable to that of PWC60 and PWC80. However, HW did not exhibit the highest thickness swelling. The PHB composites with high content of cell mass, i.e. PC and PWC80, shows larger thickness swelling than does HW and hence larger swelling coefficient (β). As we have discussed before, this larger swelling coefficient is due to weak interfacial bonding between the cell mass particles and the PHB matrix. All the properties obtained from the immersion tests were tabulated in Table IV. Overall, the PHB/WF/Cell mass composite of PWC60 shows water resistance comparable to that of the commercial WPC. The composites comprising lower cell mass content (i.e. PWC20 and 40) exhibit even better water resistance than does the commercial product.

Table IV. Comparison of water absorption properties of the extruded PHB ternary composites and the commercial HDPE composite

	HW	*PW*	*PWC20*	*PWC40*	*PWC60*	*PWC80*	*PC*
Line slope*	1.49E-04	6.63E-05	7.21E-05	1.04E-04	1.49E-04	1.63E-04	2.16E-04
MC_{sat}	22.0%	15.7%	15.1%	15.1%	16.3%	14.5%	17.5%
TS_{sat}	11.9%	7.9%	9.5%	9.8%	9.0%	13.1%	22.1%
D	2.34E-06	2.48E-06	1.80E-06	3.34E-06	5.32E-06	8.25E-06	1.04E-05
β	0.541	0.502	0.632	0.645	0.549	0.901	1.261

* $dM_t/d\sqrt{t}$, indication of water absorption rate.

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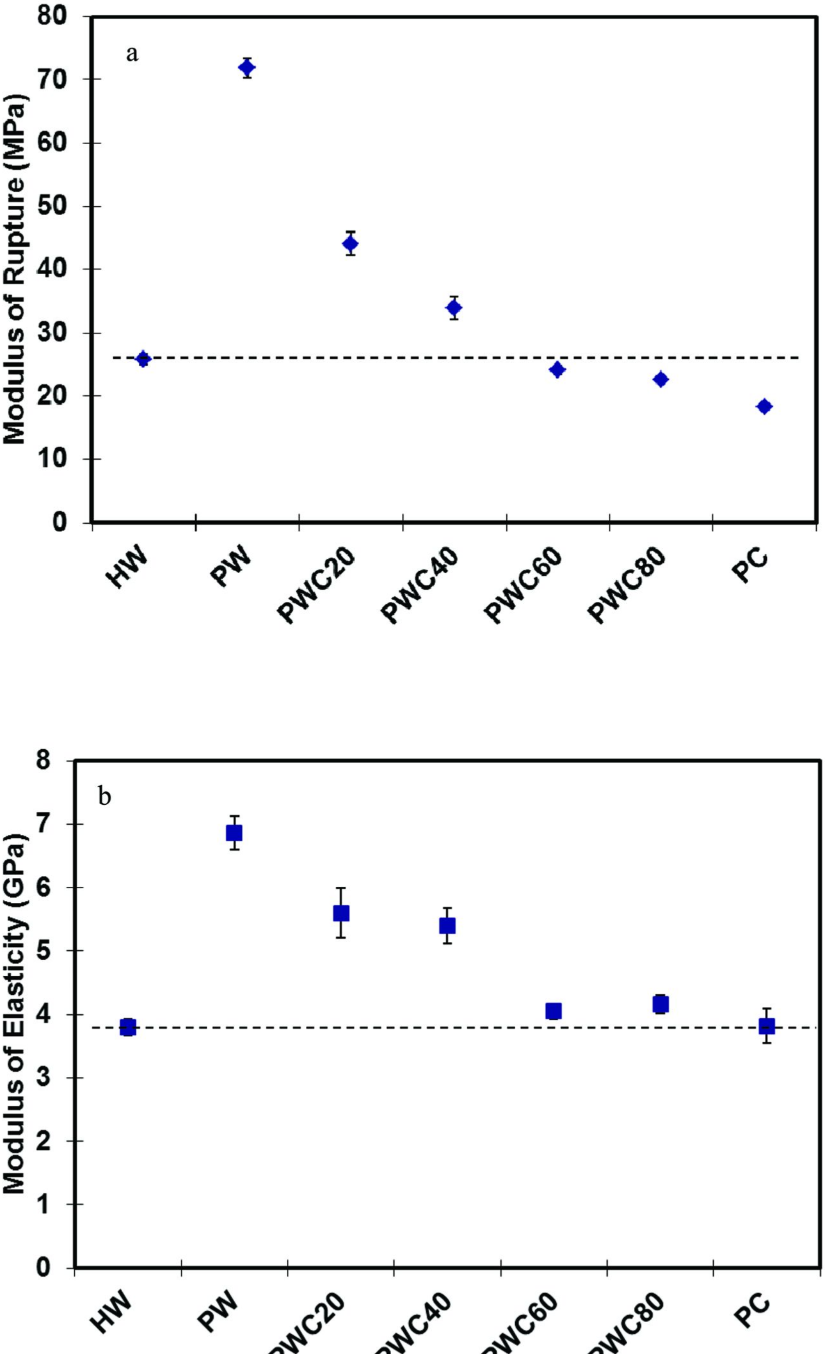

Figure 13. MOR (a) and MOE (b) of the seven composites.

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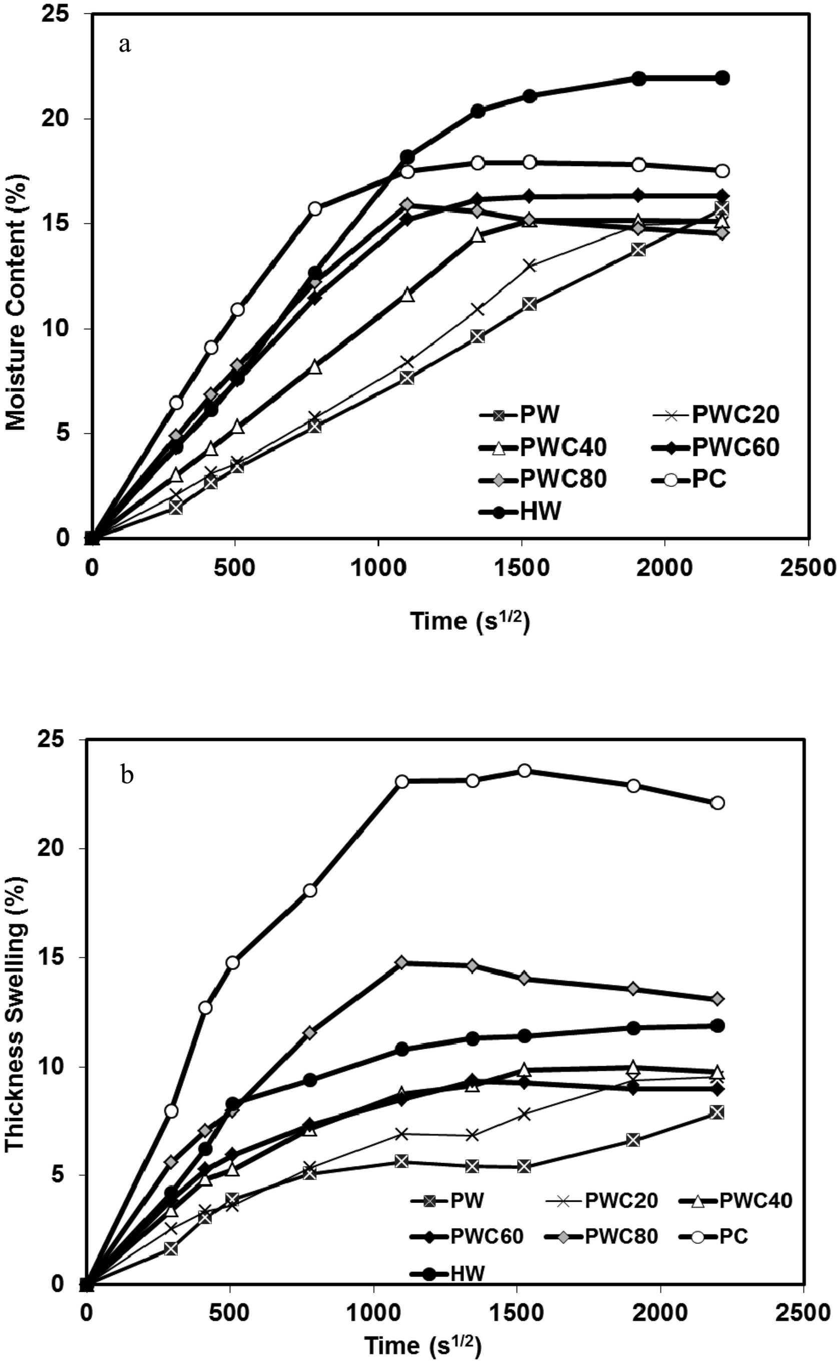

Figure 14. Moisture content (a) and thickness swelling (b) of the extruded composites.

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Conclusions

In this research we successfully developed a novel WPC comprising bacterial polyester PHB, cellulosic fiber, and cell mass. The product was developed by both extrusion-injection molding and direct extrusion. PWC60, which is primarily composed of ca. 33% PHB, 21% WF, and 32% cell mass, showed mechanical properties and water resistance comparable to those of a petroleum based commercial WPC. Moreover, this new product had great advantages in energy saving and material costs compared to petroleum based WPCs. It was found from the study that the coupling agent pMDI played a critical role in improving the mechanical properties and water resistance of the product. In direct extrusion process, a lower-than-normal extrusion temperature profile had to be used to provide adequate die pressure for material packing. This was due to the inherent low viscosity and melt strength of PHB and the presence of cell mass particles, which acted to decrease the strength further.

Acknowledgments

The authors gratefully acknowledge the financial support provided by the U.S. Department of Energy, under the grant of *Development of Renewable Microbial Polyesters for Cost Effective and Energy-Efficient Wood-Plastic Composites*.

References

1. Lee, S. Y. *Trends Biotechnol.* **1996**, *14*, 431–438.
2. Somleva, M. N.; Snell, K. D.; Beaulieu, J. J.; Peoples, O. P.; Garrison, B. R.; Patterson, N. A. *Plant Biotechnol. J.* **2008**, *6*, 663–678.
3. Terada, M.; Marchessault, R. H. *Int. J. Biol. Macromol.* **1999**, *25*, 207–215.
4. Kim, M.; Cho, K. S.; Ryu, H. W.; Lee, E. G.; Chang, Y. K. *Biotechnol. Lett.* **2003**, *25*, 55–59.
5. Ghatnekar, M. S.; Pai, J. S.; Ganesh, M. *J. Chem. Technol. Biotechnol.* **2002**, *77*, 444–449.
6. Hejazi, P.; Vasheghani-Farahani, E.; Yamini, Y. *Biotechnol. Prog.* **2003**, *19*, 1519–1523.
7. Van Hee, P.; Elumbaring, C. M. R. A.; Van der Lans, R. G. J. M.; Vander Wielen, L. A. M. J. *Colloid Interface Sci.* **2006**, *297*, 595–606.
8. Tamer, I. M.; Moo-Young, M.; Chisti, Y. *Bioprocess. Eng.* **1998**, *19*, 459–468.
9. Chen, Y.; Yang, H.; Zhou, Q.; Chen, J.; Gu, G. *Process Biochem.* **2001**, *36*, 501–506.
10. L Averous, L.; Fringant, C.; Moro, L. *Polymer* **2001**, *42*, 6565–6572.
11. Zhang, J. W.; Jiang, L.; Zhu, L. Y.; Jane, J.; Mungara, P. *Biomacromolecules* **2006**, *7*, 1551–1561.
12. Jiang, L.; Liu, B.; Zhang, J. W. *Macromol. Mater. Eng.* **2009**, *294*, 301–305.
13. Clemons, C. M. *For. Prod. J.* **2002**, *52*, 10–18.
14. Balma, D. A.; Bender, D. A. Engineering Wood Composites for Naval Waterfront Facilities, Evaluation of Bolted WPC Connections; Materials

Development, Task 2J. Project End Report; Washington State University: Pullman, WA, 2001.

15. Thompson, D. N.; Emerick, R. W.; England, A. B.; Flanders, J. P.; Loge, F. J.; Wiedeman, K. A.; Wolcott, M. P. Final Report: Development of Renewable Microbial Polyesters for Cost Effective and Energy-Efficient Wood-Plastic Composites; Department of Energy's (DOE) Information Bridge: DOE Scientific and Technical Information; 2010.
16. Coats, E. R.; Loge, F.; Wolcott, M. P.; Englund, K.; Mcdonald, A. G. *Bioresour. Technol.* **2008**, *99*, 2680–2686.
17. Rao, R. M. V. G. K.; Balasubramanian, N.; Chanda, M. *J. Reinf. Plast. Compos.* **1984**, *3*, 232–245.
18. Chowdhury, M. J. A.; Wolcott, M. P. *For. Prod. J.* **2007**, *57*, 46–53.

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Chapter 15

Modify Existing Pulp and Paper Mills for Biorefinery Operations

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The North American forest products industry has for many years been subject to low return on investment and cyclical demand for commodity products such as kraft pulp and newsprint. In the last five years, there has been a significant decline in both the kraft pulp and newsprint production due to increased competition from low cost producing countries such as Brazil and Indonesia. In order to diversify the product portfolio of the remaining mills, and possibly to revive some of those that have closed, there is renewed interest in byproducts from current operations and diversion of raw material to higher value added processes. In this chapter, we will review the most promising technologies as they could be applied to the various segments of the pulp and paper and fibreboard manufacturing industries, and attempt to predict which are most likely to be commercially viable.

1. Introduction

The North American forest products industry has for many years been subject to low return on investment and cyclical demand for commodity products such as kraft pulp and newsprint. In the last five years, there has been a significant decline in both the kraft pulp and newsprint production due to increased competition from low cost producing countries such as Brazil and Indonesia, and, in the case of

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

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newsprint, a precipitous decline in North American demand. For the Canadian manufacturing industry, this scenario has resulted in the closure of more than one quarter of the kraft mills and a similar number of newsprint mills. In order to diversify the product portfolio of the remaining mills, and possibly to revive some of those that have closed, there is renewed interest in byproducts from current operations and diversion of raw material to higher value added processes.

Historically, a number of byproducts have been manufactured at chemical pulp mills. Synthetic vanillin has been produced from kraft lignin by chemical oxidation, the solvent DMSO, tall oil and beta **sitosterol** can be made from black liquor components, and a number of kraft lignin derived preparations are still marketed by Westvaco. In the sulfite pulping field, some well established markets have been established for lignosulfonates, and fermentation products such as ethanol and Torula yeast have been and in some case continue to be manufactured. A small number of hardwood sulfite mills also sell the xylose in their spent sulfite liquor for conversion to xylitol. In terms of tonnage and revenue, these traditional byproducts represent a rather small contribution to profitability for the chemical pulping industry. In the mechanical pulping industry, which is largely newsprint, there is little current practice or history of byproduct production. However, recently knots obtained from Norway spruce have been used as raw material for production of hydroxymatairesinol, a lignan with therapeutic value. The knots can be separated from wood chips prior to pulping as knot rejects.

In recent years, a large number of potential forest biorefinery processes have been proposed. Some of these could be implemented in combination with existing pulp and paper production, while others would require larger investments, possibly at currently closed mill sites, while making use of existing infrastructure such as wood supply, raw material handling, and effluent treatment facilities.

From a resource point of view, pulp and paper is the largest existing biomass conversion industry, however, traditionally only pulp, paper, and niche chemicals have been produced as major products. The P&P manufacturing infrastructure can in principle modified or evolved into a modern biorefinery plant. Many bioconversion technologies such as biomass pretreatment and fractionation were originally derived from pulping technologies. Recent studies have shown that pulping and bleaching processes and equipment can also be adapted for hydrolysis and fermentation. Utilizing existing P&P equipment will significantly reduce the "scale-up" risk associated with the implementation of bioconversion operations (*1*). The P&P industry provides an ideal platform for the development of biofuel and bioproducts that are compatible with existing biomass harvesting, collection, transportation and processing infrastructures.

Integrating fuel and chemical production with existing P&P infrastructure will provide a promising means of mitigating production cost, improving sustainability of biomass conversion processes, and creating a valuable opportunity for forest product industries to survive and thrive.

In this chapter, we will review the most promising technologies as they could be applied to the various segments of the pulp and paper and fibreboard manufacturing industries, and attempt to predict which are most likely to be commercially viable.

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2. Opportunities for Fiberboard and Mechanical Pulping Mills

Mechanical refining is an industrial process to produce fibrous pulp from biomass, predominantly wood, by a combination of heat and mechanical force (*2*). The pulp can be processed to make either paper products such as newsprint or fiberboard products such as medium density fiberboard. Several variations of the mechanical pulping process are employed at industrial scale, including refiner mechanical pulping (RMP), thermomechanical pulping (TMP), chemithermomechanical pulping (CTMP) and chemimechanical pulping (CMP). Applying modified refining systems for steam explosion pretreatment prior to enzymatic hydrolysis for sugar production has attracted a considerable amount of interest in recent years (*3–5*). A detailed description of modified thermomechanical refining for softwood pretreatment is presented in a recent publication (*3*). As shown in Figure 1, after presteaming to soften the wood chips, they are fed to a cylinder reactor by a screw press typically having a compression ratio of up to 4:1. The feeding rate can be controlled by the rotation speed of the feeding screw. The wood chips are treated with high pressure steam in the reactor where the reaction time can be adjusted by the rotation speed of the conveyor screw in the reactor. After steam treatment, the chips are broken down by disc refining and are then blown out at high velocity through a blow line to the cyclone at atmospheric pressure. The sudden release of pressure controlled by the blow valve after disc refining creates an “explosive action” to deconstruct the fiber cell wall.

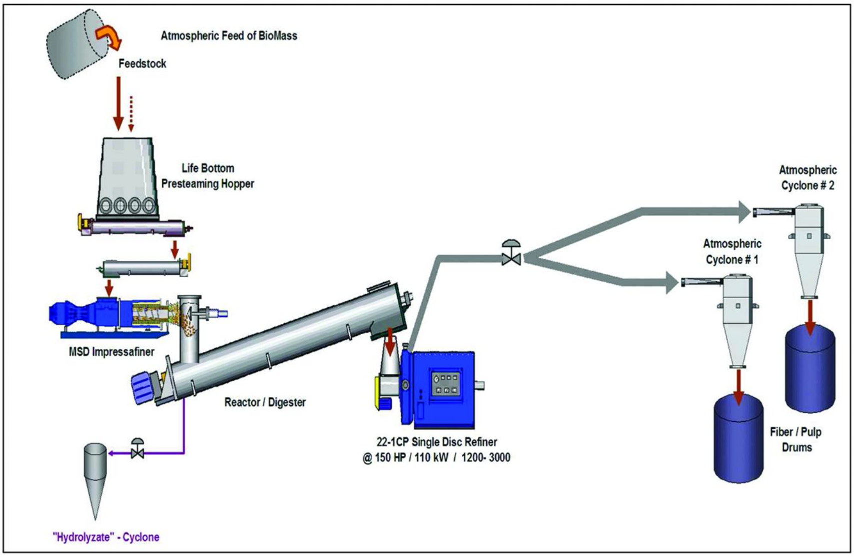
Atmospheric Feed of BioMass
Feedstock
Life Bottom
Presteaming Hopper
MSD Impressafiner
Reactor / Digester
22-1CP Single Disc Refiner
@ 150 HP / 110 kW / 1200- 3000
Atmospheric
Cyclone # 2
Atmospheric
Cyclone # 1
Fiber / Pulp
Drums
"Hydrolyzate" - Cyclone

Figure 1. Schematic diagram of a biomass pre-treatment and continuous steam explosion device. (courtesy of Andritz Inc. and FPInnovations).

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The treatment severity can be controlled by adjusting the gap distance between the refiner plates, the plate rotation speed, and the steam pressure. This refining steam explosion technology has been used for continuous pretreatment of a variety of biomass feedstocks (*4*). A patent application has claimed that hardwood substrates pretreated by continuous steam hydrolysis using a modified Andritz mechanical pulping system show markedly improved susceptibility to enzymatic hydrolysis compared to a number of pretreatment technologies such as dilute acid and ammonia fiber explosion (AFEX) (*5*). Continuous steam explosion of wheat straw has also been investigated at pilot scale. It was found that the continuous steam explosion has great advantages over batch steam explosion in achieving higher yield for both soluble sugars and water insoluble fractions. The mechanical refining effect prior to the steam explosion also effectively reduces the steam temperature and retention time required for the pretreatment. A typical mechanical pulp mill processes over 2000 tonnes per day of wood chips. Adapting thermomechanical refining for biomass pretreatment will likely provide a readily available technology for scaling up pretreatment prior to enzymatic hydrolysis of cellulose.

As well as replacing a stand-alone steam explosion vessel, mechanical refining has also been combined with modified sulfite pulping for pretreating softwood, known as sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL), developed by Zhu and Pan (*6*). A detailed description of this pretreatment method can be found in another chapter of this book.

Although Fang *et al* (*3*) have shown that refining pretreatment of spruce chips yields a significant amount of wood sugar primarily derived from hemicellulose in the water soluble fraction, a hemicellulose pre-extraction strategy may compromise the high fiber yield advantage of mechanical pulping processes for paper production. Also, partially removing hemicelluloses from mechanical pulp may be detrimental to the pulp properties. However, pre-extraction may offer a great opportunity to remove wood resins prior to refining to eliminate pitch problem encountered in the subsequent papermaking processes.

3. Opportunities for Kraft Pulp Mills

The value prior to pulping (VPP) concept has now been widely discussed as an effective approach to incorporating biorefining process elements into existing chemical pulp mill operations. Although it might be a questionable approach for high yield mechanical pulp production, VPP certainly provides a promising means of generating an additional revenue stream for chemical pulping operations. Several hemicellulose extraction schemes prior to kraft pulping have been proposed and evaluated by a number of research groups (*7–16*) Using various acids, pulping liquor and water to extract wood hemicellulose from biomass materials prior to pulping has been extensively investigated by the pulp and paper industry (*17*). While the main focus of the early studies was on producing high quality viscose rayon grade pulp, opportunities for converting hemicellulose streams to ethanol and other chemicals have also been explored (*18*, *19*). Probably the most significant recent achievement in this area at an industrial scale is the implementation of a wood pre-hydrolysis process at the AV Nackawic hardwood kraft dissolving pulp mill located in New Brunswick, Canada. Significant pilot scale studies carried out at FPInnovations in collaboration with University of New Brunswick contributed to the successful implementation of this process. The wood pre-hydrolysis step is incorporated into the cycle of batch chemical cooking of wood chips (*20*). As illustrated in Figure 2, the wood chips are loaded into the digester, and steam is then introduced to heat the wood chips to a temperature of about 170°C in order to start the prehydrolysis. After hydrolysis, the hydrolysate can be either displaced by neutralizing liquor or simply drained from the digestor. The kraft cooking liquor is then introduced into the digester to perform the pulping stage. The effects of various pre-hydrolysis conditions on hemicellulose extraction and final pulp quality have recently been investigated (*21*). It was found that water/steam hydrolysis of hardwood chips produces a hydrolysate with higher oligomeric sugar content, while acid catalyzed prehydrolysis (e.g. H_2SO_4, SO_2) will produce sugar monomers in high yield. SO2 catalyzed prehydrolysis also produces a hydrolysate enriched with low molecular weight phenolic compounds (Figure 3). It was concluded that hemicellulose extraction can be readily implemented in hardwood dissolving pulp mills while the application of the same process in paper-grade pulp mills require more sophisticated **control of both hydrolysis and pulping conditions.** The technical and economic feasibility of incorporating a hemicellulose extraction process in a dissolving pulp mill to produce biofuel and biochemicals such as ethanol, xylitol and furfural has been explored. The effect of mill operational conditions on the composition of pre-hydrolysate has been shown in a previous publication (*20*). As steam is used in the mill process to extract hemicellulose rather than hot water, wood hydrolysate with much higher sugar concentrations can be obtained. The high sugar concentration will likely improve the efficiency of separation and fermentation processes for bioproducts development.

4. Adapting Pulping Equipment for Enzymatic Hydrolysis and Fermentation

Applying pulping equipment as currently found in pulp mills for improving enzymatic hydrolysis and fermentation has also been explored previously. We have clearly demonstrated the benefit of using a peg mixer typically employed in pulp bleaching (Figure 4) to achieve a high cellulose-to-glucose conversion yield at high substrate consistencies (up to 30%) (*22*). A typically industrial peg mixer consists of tubular vessels having one of two shafts attached with pegs. The rotation of the pegs creates shear actions to move the pulp/substrate. The shear forces constantly expose new fiber surface to contact with chemicals to allow a thorough mixing and optimized reactivity. During enzymatic hydrolysis of lignocellulosic substrates, the effective transfer of enzymes to the reactive sites on fiber cellulose has a major impact on the hydrolysis rate. According to the model developed by Osawa and Schuerch (*23*) (Figure 5), at low substrate consistencies (<1% w/v), enzymes are effectively transported by the exterior force of shaking or agitation to the reaction site of the fiber by convection across the mobile

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water layer (d_1) and by diffusion across the immobile water layer (d_2) adjacent to the fiber surface (*23*, *24*). At low consistency, the immobile water layer is of maximum thickness. Diffusion across d_2 is the rate-determining step because convective transport across d_1 is faster. As the consistency is increased from low to medium (about 10%), the mobile water layer is progressively eliminated leaving only the immobile layer. The thickness of water layer determines the rate. It has been suggested that in a high-intensity mixing system, fluidization of a fiber suspension can effectively set the immobile water layer d_2 in motion (*25*, *26*) so that the mass can be transported by convection instead of the more sluggish diffusion process. In the high consistency range (>20%), only a thin immobile water layer envelopes the fiber and most of the water is confined within the fiber, thus considerably decreasing the diffusion path length of enzyme to the fiber. Due to the disappearance of the mobile layer, enzymes cannot freely disperse to all fiber surfaces and most of them are concentrated in the smaller area of the fiber aggregates.

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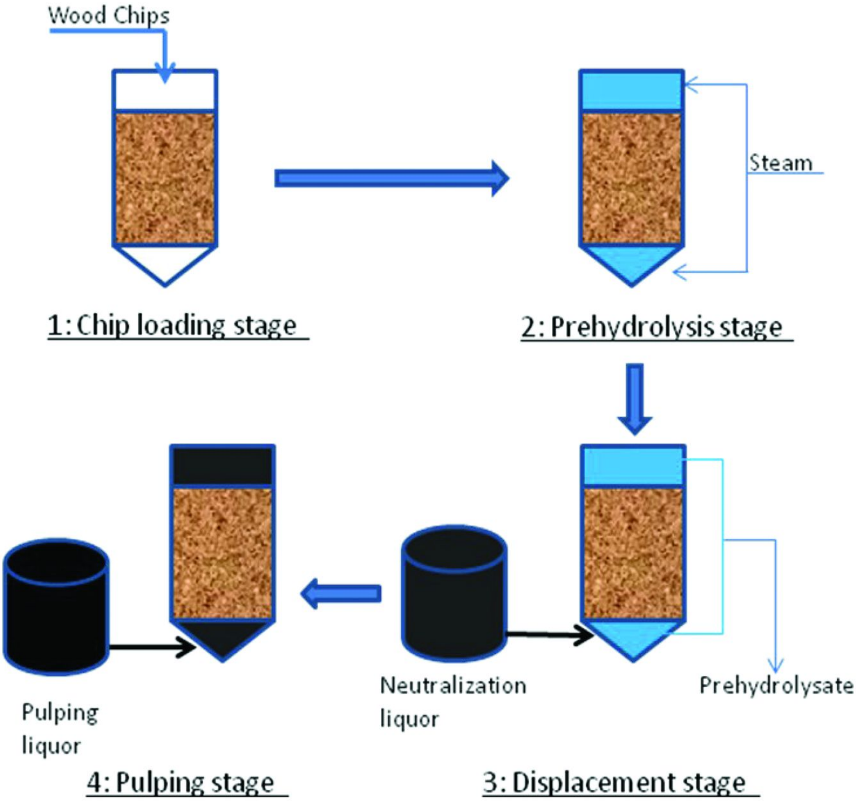

Figure 2. Schematic diagram of wood prehydrolysis process.

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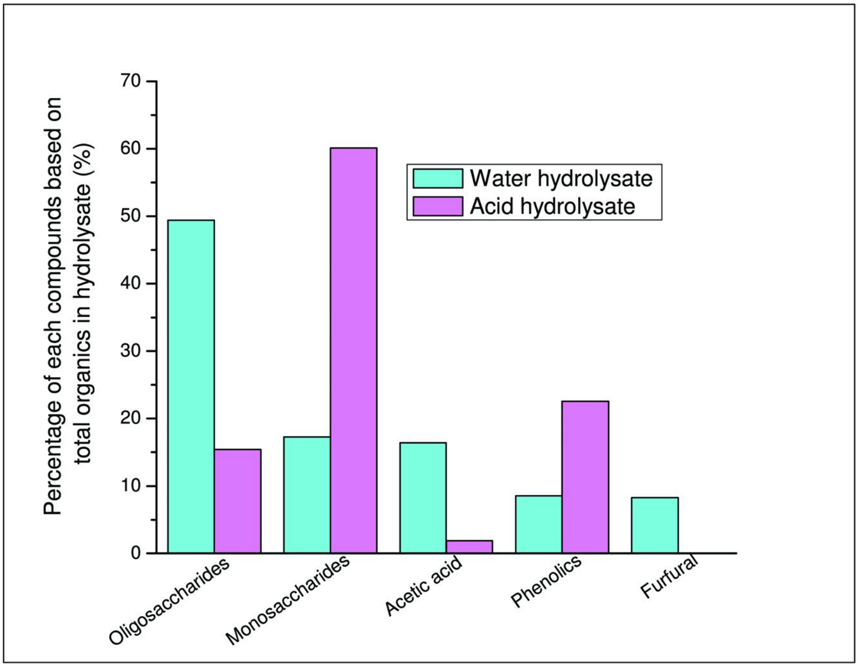

Figure 3. The effect of different prehydrolysis chemistries on the chemical composition of prehydrolysates.

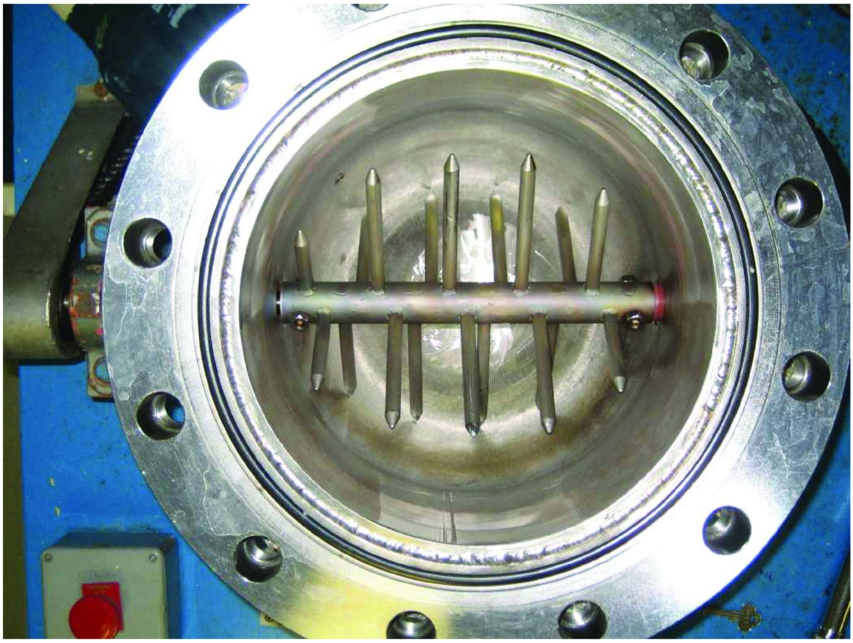

Figure 4. The inner chamber of a laboratory peg mixer (adapted from (22)).

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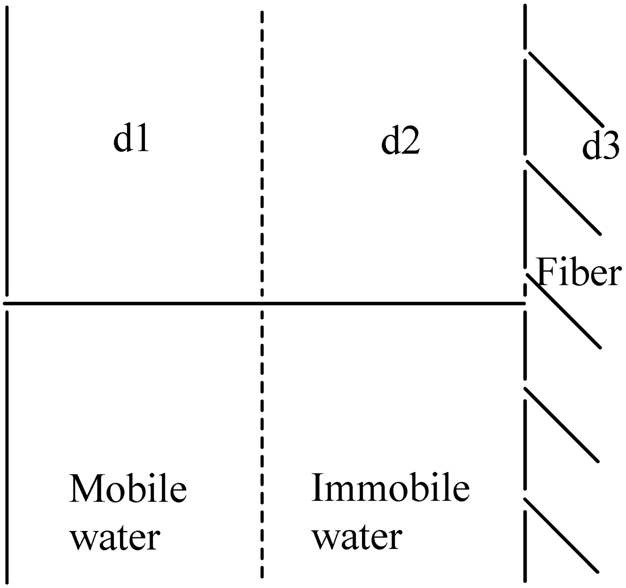

Figure 5. Mass transfer process model (adapted from (23)).

The ability of a peg mixer to continuously expose the fiber surfaces to the enzyme is the main reason that this type of equipment can be readily applied for high consistency hydrolysis of lignocellulosic biomass. Hydrolysis of unbleached softwood kraft pulp (UBSW) has also been evaluated in a peg mixer at 2% and 20% substrate consistencies. The monosugars contained in the UBSW hydrolysate obtained from enzymatic hydrolysis at 20% consistency were mainly glucose, xylose and mannose, with trace amounts of arabinose and galactose. As shown in Figure 6, the UBSW is easily hydrolyzed by enzymes at 2% substrate consistency. The substrate released ~18 g/L of glucose after 24 h enzymatic hydrolysis (Figure 6A) which represents a complete conversion of all cellulose to glucose (Figure 6B). Hydrolysis of UBSW at 20% substrate consistency also yielded a high glucose concentration. The glucose content reached 140 g/L in the hydrolysate after 96 h of enzymatic hydrolysis, corresponding to a cellulose to glucose conversion yield of about 80%. Compared to hydrolysis of UBHW at 20% consistency, UBSW had a lower cellulose-to-glucose conversion rate which may due to the higher lignin content. Maekawa (1996) previously reported that softwood enzymatic hydrolysis is less efficient due to the more recalcitrant lignin. To demonstrate the feasibility of incorporating a complete lignocellulosic biomass-to-biofuel and bioproducts conversion in a mill pulp, a large scale pilot trial was carried out at FPInnovations Laboratory in Pointe Claire, Quebec (*27*). The hydrolysis experiments were carried out in two pilot scale vessels of 400L and 6000L capacity, which are typically used as pilot scale pulp bleaching. These sizes were chosen to represent a scaled down version of a targeted pulp mill which already has fermentation facilities. Fermentation of wood sugars to ethanol is not a new subject to the pulp and paper industry. A number of pulp mills have had decades of experience in producing ethanol for various commercial applications from wood sugars. Pilot scale pulp washing, draining and conveying equipment were also used in this study. The pilot trial results have proven the feasibility of incorporating cellulosic residue hydrolysis into existing mill operations. Capital cost can be minimized and potential synergies achieved by using mill water and steam for substrate washing, detoxification, and product drying. Based on daily averages, a 1000 t/d chemical pulp mill yields 20-30 tonnes of knot residues. Effective hydrolysis of these residues requires approximately 40-200 million FPU of cellulase enzymes per day. The market price of commercial cellulase enzyme is likely a major factor influencing the economic feasibility of knots to biofuel conversion technology.

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5. Producing High Value Products from Pulp Mills

One of the major concerns facing the P&P industry in moving toward biofuel production is the fear of another "commodity trap". P&P has been sustained by the production of huge volumes of commodity products to offset the low profit margins. Although biofuel is likely to have a very large market, the enormous capital cost and sophisticated value chain infrastructure pose unpredictable economic risks. One effective and practical way to alleviate these risks is to develop high value products along with the biofuel production. The P&P industry probably has the richest experience among all existing industries in producing high value chemicals from lignocellulosic biomass material; manufacturing of silvichemicals has been practiced for over a century. Processes for separating tall oil and turpentine as commercial products from black liquor have been in place for decades and are still widely used in many mills. It has long been possible to extract phytochemicals from tree bark and knots. Plant-derived lignans and flavonoids have been widely accepted and commercialized as nutraceutical products. Devising an integrated process to produce biofuel and bioproducts will undoubtedly enhance the sustainability of a biomass refinery. Perhaps one of the greatest opportunities exists in utilizing biomass lignin. The invention of the Tomlinson recovery boiler to recycle chemicals and recover energy from lignin has been the key technology for the kraft industry at least up until today. It is likely the role of lignin as fuel will be diminished in the new biomass refinery industry. Instead, lignin will become a major new source for producing bioproducts and biochemicals.

Lignin is the largest source of renewable material with an aromatic skeleton. Depolymerizing lignin to low molecular weight aromatic and phenolic compounds offers attractive opportunities to produce a range of high value chemicals (*28*). Lignin can be depolymerized into smaller molecular weight phenolic compounds by a number of chemical and/or biological means (*29*). Many types of monomeric and oligomeric subunits of lignin are recognized for their antimicrobial, anti-oxidation, and anticorrosive properties (*30–34*). Traditional pulping and bleaching technologies have been developed to reduce and eliminate lignin from cellulose matrices (*35*, *36*). The wealth of knowledge accumulated in delignification chemistry will provide new research avenues for the development of economically feasible lignin conversion technology. Catalytic

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depolymerization of lignin to low molecular weight phenolic compounds represents a promising opportunity to expand lignin derived markets which will in turn greatly improve the economics of biomass-to-advanced biofuel conversion processes. As a primary structural component of fiber cell wall, cellulose provides

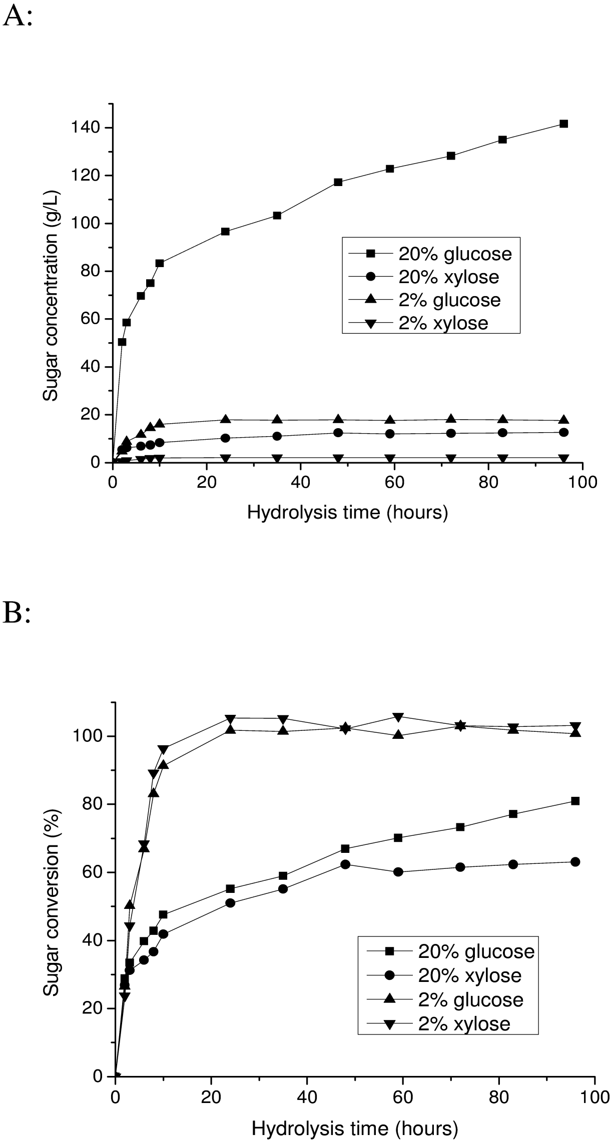

Figure 6. Hydrolysis of unbleached softwood kraft pulp (UBSW) at 2% and 20% substrate consistency in a peg mixer, based on A) monosaccharide concentration formed and B) percent sugar conversion.

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lignocellulosic biomass with strength and rigidity. The orderly structured cellulose crystals offer a new class of nanomaterials which can be manufactured directly from a pulp mill (*37–39*). The properties and potential application of nanocrystalline cellulose are discussed in detail in another chapter of this book.

6. Future Directions and Recommendations

Integrating a biomass refinery with P&P manufacturing provides a logical pathway towards a more sustainable forest products industry. It creates a new paradigm in biorefinery research and development strategies. The technologies developed over many years for mechanical and chemical pulping can be adapted to provide pathways to new or less costly bioproducts. The flourishing of the P&P industry has not only brought a vibrant economy and prosperity to North America for over a century, but has also stimulated the quest for better knowledge and understanding of plant materials resulting in new scientific disciplines. The wealth of knowledge accumulated which has provided the backbone for P&P industry will immensely valuable to in the development of forest-based biorefinery. Our journey towards developing a sustainable bioeconomy is at a crossroads. While scientists and other stakeholders are searching for revolutionary ideas to solve the biomass recalcitrance, in fact wood chemistry may already provide a bank of information. Capitalizing on knowledge and wisdom accumulated from developing modern P&P technology will benefit research and development, and deployment of a biomass conversion industry enormously.

Canadian hardwood kraft mills are a good example of where an integrated biorefinery should be considered. The addition of a prehydrolysis step can allow these mills to manufacture dissolving grade pulps and delivers a pentose-rich stream for manufacturing of byproducts such as furfural, xylitol, or fermentation products. One mill in Eastern Canada is already doing this and another is in the process of conversion. Depending on the market for dissolving grades, several other mills could move in this direction, or alternatively, depending on the price of transportation fuels, part of the cellulose could be converted to ethanol.

A flexible product portfolio can also be achieved by adaption of mechanical pulping technologies so that either fibrous products or bioproducts can be manufactured. As discussed in this chapter, thermomechanical pulping processes for paper and fibreboard manufacturing are similar to the steam pretreatment processes developed for pretreatment of cellulose prior to hydrolysis and fermentation to biofuels. In theory, equipment can be designed to operate at different temperatures and pressures for integrated manufacturing of fibrous and biofuel products, depending on market demand. Major mechanical pulping equipment manufacturers have recognized this opportunity and are currently working on new refiner processes for bioconversion of forest based material. When combined with the advances in enzyme and fermentation technologies which have been reported, there should be new opportunities for manufacturing of chemicals and fuels from sustainable raw materials.

Given the huge investment in forest resource infrastructure and current inventory of unprofitable or disused mills, the timing appears to be right for a

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new resource industry which like the petrochemical refinery, can produce a range of products by making use of flexible manufacturing technologies. Progressive modification of P&P mills to produce a wide spectrum of bioproducts sheds a bright light to the future of this “sunset” industry and, at the same time, provides opportunities for new biorefinery products.

References

1. Cort, J. B.; Pschorn, T.; Stromberg, B. Minimize scale-up risk. *Chem. Eng. Prog.* **2010**, *106* (3), 39–49.
2. Smook, G. A. *Handbook for Pulp and Paper Technologists*, 3rd ed.; Angus Wilde Publications, Inc.: Vancouver, 2002.
3. Fang, H. X.; Deng, J.; Zhang, T. Dilute acid pretreatment of black spruce using continuous steam explosion system. *Appl. Biochem. Biotechnol.* **2010**. http://dx.doi.org/10.1007/s12010-010-9061-6.
4. Pschorn, T.; Sabourin, M.; Mraz P. High Pressure Compressor and Steam Explosion Pulping Method. U.S. Patent 12/057,409, 2008.
5. South, C. R.; Garant, H.; Martin, R. L. Combined Thermochemical Pretreatment and Refining of Lignocellulosic Biomass. WO Patent 2008/131229 A1. US, 2008.
6. Wang, G. S.; Pan, X. J.; Zhu, J. Y.; Gleisner, R.; Rockwood, D. Sulfite pretreatment to Overcome recalcitrance of lignocellulose (SPORL) for robust enzymatic saccharification of hardwoods. *Biotechnol. Prog.* **2009**, *25* (4), 1086–1093.
7. Chen, X. W.; Lawoko, M.; van Heiningen, A. Kinetics and mechanism of autohydrolysis of hardwoods. *Bioresour. Technol.* **2010**, *101* (20), 7812–7819.
8. Frederick, W. J.; Lien, S. J.; Courchene, C. E.; DeMartini, N. A.; Ragauskas, A. J.; Iisa, K. Co-production of ethanol and cellulose fiber from Southern Pine: A technical and economic assessment. *Biomass Bioenergy* **2008**, *32* (12), 1293–1302.
9. Huang, H. J.; Ramaswamy, S.; Al-Dajani, W. W.; Tschirner, U. Process modeling and snalysis of pulp mill-based integrated biorefinery with hemicellulose pre-extraction for ethanol production: A comparative study. *Bioresour. Technol.* **2010**, *101* (2), 624–631.
10. Jin, Y. C.; Jameel, H.; Chang, H. M.; Phillips, R. Green liquor pretreatment of mixed hardwood for ethanol production in a repurposed kraft pulp mill. *J. Wood Chem. Technol.* **2010**, *30* (1), 86–104.
11. Liu, S. J.; Mishra, G.; Amidon, T. E.; Gratien, K. Effect of hot-water extraction of woodchips on the kraft pulping of eucalyptus woodchips. *J. Biobased Mater. Bioenergy* **2009**, *3* (4), 363–372.
12. Mao, H. B.; Genco, J. M.; van Heiningen, A.; Pendse, H. Kraft mill biorefinery to produce acetic acid and ethanol: Technical economic analysis. *Bioresources* **2010**, *5* (2), 525–544.
13. Sixta, H.; Borgards, A. New technology for the production of high-purity dissolving pulps. *Papier* **1999**, *53* (4), 220–234.

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14. Tunc, M. S.; Lawoko, M.; van Heiningen, A. Understanding the limitations of removal of hemicelluloses during autohydrolysis of a mixture of southern hardwoods. *Bioresources* **2010**, *5* (1), 356–371.
15. Tunc, M. S.; van Heiningen, A. R. P. Hemicellulose extraction of mixed southern hardwood with water at 150 degrees C: Effect of time. *Ind. Eng. Chem. Res.* **2008**, *47* (18), 7031–7037.
16. Yoon, S. H.; Cullinan, H. T.; Krishnagopalan, G. A. Reductive modification of alkaline pulping of Southern Pine, integrated with hydrothermal pre-extraction of hemicelluloses. *Ind. Eng. Chem. Res.* **2010**, *49* (13), 5969–5976.
17. Rydholm, S. *Pulping Processes*; John Wiley & Sons: New York, 1965.
18. Springer, E. L. Prehydrolysis of hardwoods with dilute sulfuric acid. *Ind. Eng. Chem. Prod. Res. Dev.* **1985**, *24* (4), 614–623.
19. Elmore, C. L. Method of Producing Kraft Pulp Using an Acid Prehydrolysis and Pre-extraction. U.S. Patent 4,436,586, 1984.
20. Saeed, A.; Jahan, M. S.; Li, H.; Zahua, L, Ni Y., van Heiningen, A. Mass balances of components dissolved in the pre-hydrolysis liquor of kraft-based dissolving pulp production process from canadian hardwoods. *Biomass Bioenergy* **2010**, DOI:10.1016/j.biombioe.2010.08.039, http://dx.doi.org/10.1016/j.biombioe.2010.08.039.
21. Radiotis, T., Zhang, X., Paice, M. G., Byrne, V. Optimizing Production of Xylose and Xylooligomers from Wood Chips; PInnovations Research Report PRR 1929; 2010.
22. Zhang, X.; Qin, W. J.; Paice, M. G.; Saddler, J. N. High consistency enzymatic hydrolysis of hardwood substrates. *Bioresour. Technol.* **2009**, *100* (23), 5890–5897.
23. Osawa, Z.; Schuerch, C. The action of gaseous reagents on cellulosic materials. I: Ozonization and reduction of unbleached kraft pulp. *Tappi J.* **1963**, *46* (2), 79–89.
24. Bouchard, J.; Nugent, H. M.; Berry, R. M. The role of water and hydrogen-ion concentration in ozone bleaching of kraft pulp at medium consistency. *Tappi J.* **1995**, *78* (1), 74–82.
25. Laxen, T.; Ryynanen, H.; Henricson, K. Medium-consistency ozone bleaching. *Pap. Puu* **1990**, *72* (5), 504–507.
26. Reeve, D. W.; Earl, P. F. Mixing gases, water, and pulp in bleaching. *Tappi J.* **1986**, *69* (7), 84–88.
27. Zhang, X.; Tu, M. B.; Paice, M.; Sacciadis, G.; Jiang, Z. H.; Jemaa, N.; et al. Bioconversion of knot rejects from a sulphite pulp mill to ethanol. *Bioresources* **2010**, *5* (1), 23–42.
28. Whiting, D. A. Natural Phenolic compounds 1900-2000: A bird's eye view of a century's chemistry. *Nat. Prod. Rep.* **2001**, *18* (6), 583–606.
29. Holladay J. E., Bozell J. J.; White J. F.; Johnson D. Top Value-Added Chemicals from Biomass: Volume II—Results of Screening for Potential Candidates from Biorefinery Lignin; Contract No. DE-AC05-76RL01830, Document No. PNNL-16983; U.S. Department of Energy, 2007.
30. Nicholson, R. L.; Hammerschmidt, R. Phenolic compounds and their role in disease resistance. *Annu. Rev. Phytopathol.* **1992**, *30*, 369–389.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch015

31. Higuchi, T. Biodegradation of lignin: Biochemistry and potential applications. *Experientia* **1982**, *38* (2), 159–66.
32. Raja, P. B.; Sethuraman, M. G. Natural products as corrosion inhibitor for metals in corrosive media: A review. *Mater. Lett.* **2008**, *62* (1), 113–116.
33. Baurhoo, B.; Phillip, L.; Ruiz-Feria, C. A. Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. *Poult. Sci.* **2007**, *86* (6), 1070–1078.
34. Baurhoo, B.; Ruiz-Feria, C. A.; Zhao, X. Purified lignin: Nutritional and health impacts on farm animals: A review. *Anim. Feed Sci. Technol.* **2008**, *144* (3−4), 175–184.
35. Gierer, J. Chemistry of delignification. 1. General concept and reactions during pulping. *Wood Sci. Technol.* **1985**, *19* (4), 289–312.
36. Gierer, J Chemistry of delignification. 2. Reactions of lignins during bleaching. *Wood Sci. Technol.* **1986**, *20* (1), 1–33.
37. Hamad, W. Y.; Hu, T. Q. Structure−process−yield interrelations in nanocrystalline cellulose extraction. *Can. J. Chem. Eng.* **2010**, *88* (3), 392–402.
38. Domtar and FPInnovations Join Forces to Develop Innovative Fiber-based Nanotechnologies at New Demonstration Plant. FPInnovations, 2010. http://www.fpinnovations.ca/pdfs/BinderEn.pdf (accessed September 29, 2010).
39. Habibi, Y.; Lucia, L. A.; Rojas, O. J. Cellulose Nanocrystals: Chemistry, self-assembly, and applications. *Chem. Rev.* **2010**, *110* (6), 3479–3500.

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Chapter 16

Integrated Forest Biorefinery − Sulfite Process

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The majority of lignin and a part of the hemicelluloses in wood chips are dissolved during the sulfite pulping process. The spent liquor of sulfite pulping process contains a significant amount of lignocellulosic materials, which can be converted to various value-added products. Such a process can fit into the forest biorefinery concept very well. In this chapter, the processes configurations of biorefinery commercially relevant to various sulfite pulping processes are discussed. Additionally, the production of various value-added products and the compatibility of these processes to the existing facilities and configuration of sulfite pulping processes are included.

Introduction

Sulfite pulping process has been practiced for the pulp production for more than a century (*1*, *2*). In this process, lignin and hemicelluloses are dissolved in the sulfite spent liquor (SSL) and removed from the pulp (solid residue). The SSL contains a significant amount of dissolved organics, which can be converted to several value-added products. The utilization of the lignocellulosic-rich SSL for the production of various value-added products has been practiced commercially. However, the research and development activities are still on-going due to the complexity involved in recovering and converting the dissolved lignocellulosic materials of the SSL to these desired products.

Today, the sulfite pulping process is used for the production of paper- or dissolving-grade pulps. Approximately, 60% of dissolving pulps are produced via the sulfite pulping process (*3*). In the production of dissolving pulp, it is crucial to design various process steps so that the maximum efficiency in removing

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the lignin and hemicelluloses are achieved (*2*). Similarly, the acidic environment of the sulfite pulping process for paper-grade pulp production results in a major dissolution of hemicelluloses and lignin during pulping. The hardwood SSL of paper-grade pulping contains 15-22% sugars (*4*, *5*), and 50-65% lignosulfonate (based on the total solids) (*6*). Similarly, the chemical pretreatment of wood chips in the production of chemimechanical pulp, e.g. CTMP or semichemical pulp, such as NSSC, causes lignin and hemicelluloses dissolution in the spent liquor.

Due to the more severe pulping conditions, the SSL from the dissolving pulp-grade sulfite pulping is richer in ligocellulosic materials than that from the paper-grade sulfite pulping. These lignocellulosic materials in the SSLs can be employed in the production of various value-added products. In this case, instead of burning the organics for the energy production or treating them in the costly wastewater treatment system of mills, various value-added products can be extracted from the SSL.

Alternatively, the sulfite treatment, together with mechanical treatment, can be the basis for a new process called the SPORL process (*7–9*). In such a process, ethanol is the major product, and pulp is no longer a product.

In this chapter, the biorefinery units commercialized or proposed for various sulfite pulping processes are comprehensively described; and the process characteristics, commercial relevance and other aspects related to the forest biorefinery are discussed.

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Biorefinery Possibilities for Dissolving Pulp- and Paper-Grade Sulfite Pulping Process

Integrated Forest Biorefinery for Ammonium-Based Sulfite Pulping Process for Producing Dissolving Pulps

The spent sulfite liquor (SSL) of the dissolving pulp production can be utilized to produce various value-added products. Figure 1 shows a commercialized integrated forest biorefinery unit for ammonium-based sulfite process for dissolving pulp production (*10*, *11*).

As can be seen, the main cellulosic product of this process is dissolving pulp. The SSL of this process is concentrated from 12% (wt.) to 22% prior to fermentation via evaporator-I. This evaporation not only increases the concentration of sugars for the fermentation, but also reduces the amount of potentially toxic volatile sulfur compound (SO_2). The volatile sulphur compounds are carried off in the evaporators for subsequent sulphur recovery. Ethanol is produced in a continuous-flow cascade fermentor with a retention time of 24 h using *S. cerevisiae*. After the fermentation, the residual liquor is concentrated in Evaporator-II to 50% wt. A part of the concentrated solution can be marketed as liquid lignin, which can be used as animal feed, binders or highway dust control agent. Another portion can be spray-dried and marketed as lignosulfonates used for other value-added applications. The remainder is sent to the recovery process (*11*). Additionally, a part of effluent of the mills can be sent to the anaerobic effluent treatment system to generate biogas. The generated biogas is first scrubbed to purify methane, and then sent to the recovery boiler to supply a part of the fuel requirement of the mill. The scrubber operating unit may include Claus type reactor, which will convert the produced H_2S to sulphur, another by-product of the plant (*10*).

Integrated Forest Biorefinery for Magnesium-Based Sulfite Pulping Process for Producing Paper-Grade Pulps

The hemicelluloses are essential for providing a good hydrogen bonding capacity of the pulp, thus the strength for paper-sheets. Therefore, the sulfite pulping process for the production of paper-grade pulps is usually performed in such ways that a significant amount of hemicelluloses is still present in cellulose fibers. Magnefite or magnesium-based sulfite pulping process was designed for the production of paper-grade pulps.

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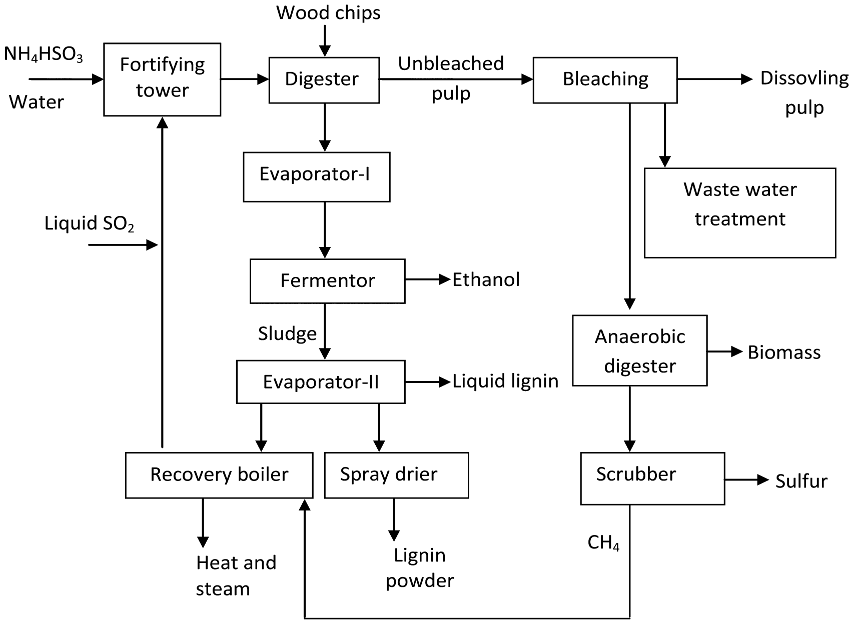
Wood chips
NH_4HSO_3
Water
Fortifying tower
Digester
Unbleached pulp
Bleaching
Dissovling pulp
Evaporator-I
Waste water treatment
Liquid SO_2
Fermentor
Ethanol
Sludge
Evaporator-II
Liquid lignin
Anaerobic digester
Biomass
Recovery boiler
Spray drier
Scrubber
Sulfur
Heat and steam
Lignin powder
CH_4

Figure 1. Process diagram of a biorefinery unit based on ammonia-based sulfite dissolving pulp production process. (Reproduced with permission from reference (11). Copyright 1993 Springer).

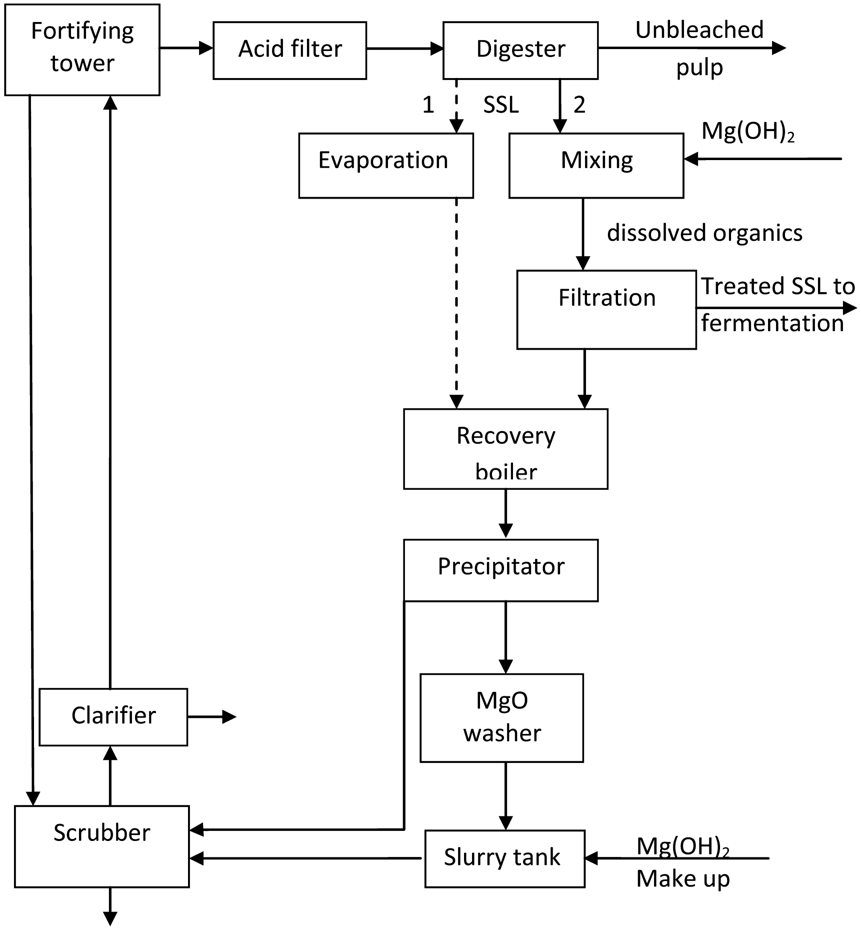
Fortifying tower
Acid filter
Digester
Unbleached pulp
1
SSL
2
Evaporation
Mixing
$Mg(OH)_2$
dissolved organics
Filtration
Treated SSL to fermentation
Recovery boiler
Precipitator
MgO washer
Clarifier
Scrubber
Slurry tank
$Mg(OH)_2$ Make up

Figure 2. Stream 1: the magnesium-based sulfite process for the production of paper grade pulp, Stream 2: proposed process diagram for a magnesium-based biorefinery unit. (Reproduced with permission from reference (13). Copyright 1980 John Wiley & Sons).

The SSL of magnesium-based sulfite pulping process can be used to produce value-added products. In the case of the hardwood SSL, the SSL contains a significant amount of xylan (50-70% of the total sugars in SSL), which could potentially be converted to ethanol, but the inhibitors should be eliminated prior to ethanol production (*5*, *12*). Therefore, extra purification (detoxification) steps may be required. In the case of softwood SSL, the SSL may contain a significant amount of lignin (*4*). This lignin can be separated and sold as a product. The high amount of hexose sugars present in the SSL would facilitate the ethanol production via several hexose-fermenting yeasts used in industry. Figure 2 shows the magnesium-based sulfite process for the production of paper-grade pulp (Stream 1) (*13*), and proposed process diagram for a magnesium-based biorefinery unit (Stream 2).

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It is noted that the SSL is separated from the pulp and then $Mg(OH)_2$ is added to the SSL (Stream 2), which is used in the existing configuration of magnesium-based sulfite pulping process. This liming process (via adding $Mg(OH)_2$) would adjust the pH of the SSL for the downstream biorefinery stages to produce ethanol, for example. The precipitation of some organics from the pH adjustment process would also serve for decreasing the inhibitor concentration of the SSL. The precipitates are sent to the recovery boiler of the mill, in which the lignosulfonates supply a part of the heat/electricity requirement of the mill. A part of SSL may be sent via evaporation to the boiler in stream 1(open line in Figure 2). The subsequent stages of this biorefinery unit resemble the stages existing in the magnesium-based sulfite process. The treated SSL can be further utilized for value-added production.

Composition of the SSL Produced in Various Sulfite Pulping Processes

SSL of Dissolving Pulp-Grade Process

The compositions of the SSL produced from hardwood and softwood in the ammonia-based dissolving pulp production is listed in Table I.

No arabinose was found in any of the SSL samples listed in Table I. Evidently, the SSL of hardwood contained a significant amount of xylose, whereas the SSL of softwood contained a considerable amount of mannose. This is a basic feature of the wood chemistry of the two species. It is therefore important that the biorefinery design reflects the porposed wood supply, which is intended to be used in the mill. The conversion process of hemicelluloses to ethanol, for example, must be optimized for either wood species. The concentration of acetic acid was much higher in the hardwood SSL than in the softwood SSL (*11*). The acetic acid can be recovered, especially from the hardwood SSL, as a by-product of this process.

In another laboratory study, beech and spruce wood species were cooked for the dissolving pulp production. The compositions of the SSL produced are listed in Table II (*2*).

It is noted that xylan and mannose were the major hemicelluloses of hardwood and softwood SSL, respectively. Under strong acidic conditions of sulfite pulping, some other sugar related products, such as furfural, xylonic acid, mannonic acid, are also present in the SSL (*14*).

SSL of Paper-Grade Process

The compositions of the SSL from magnesium sulfite pulping of beech wood for paper-grade pulp production are listed in Table III (*2*).

Table I. Composition of hardwood and softwood SSL obtained from an Eastern Canada mill at a concentration of 20% (wt.). (Reproduced with permission from reference (*11*). Copyright 1993 Springer)

	Xylose, g/l	*Mannose, g/l*	*Glucose, g/l*	*Galactose, g/l*	*Reducing sugars, g/l*	*Acetic acid, g/l*
Hardwood SSL, No 1	20.8	7.1	1.8	-	29.7	9.3
Hardwood SSL, No 2	24	6.5	3	-	33.5	7.6
Softwood SSL	8.3	17.1	6.3	3.7	35.4	2.7

Table II. The SSL compositions of beech and spruce for the production of dissolving pulp at an intrinsic viscosity of 700 ml/g. (Reproduced with permission from reference (*2*). Copyright 2006 WILEY-VCH)

Component	*Beech*	*Spruce*
Xylose, g/kg[1]	100.9	18.4
Mannose, g/kg	7	58.1
Glucose, g/kg	8.4	19.8
Galactose, g/kg	4.8	n.d.[2]
Arabinose, g/kg	1.6	0.6
Rhamnose, g/kg	2.8	<0.4
Furfural, g/kg	9.8	2.3
Acetic acid, g/kg	61.7	26.8
Xylonic acid, g/kg	29.6	15.5
Mannonic acid, g/kg	<0.5	20.2

[1] g/kg on oven dried wood. [2] not detected.

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Table III. Dissolved wood components and their degradation products in magnesium sulfite cooking of beech wood. (Reproduced with permission from reference (*2*). Copyright 2006 WILEY-VC0H)

H-factor	*Dissolved carbohydrate, %*	*Dissolved lignin, %*	*Xylose, %*		*Arabinose, %*		*Glucose, %*		*Mannose, %*		*Galactose, %*		*Furfural, %*	*Acetic acid, %*	*Xylonic acid, %*	*Methanol, %*
			Mon[1]	Oli[2]	Mon	Oli	Mon	Oli	Mon	Oli	Mon	Oli				
18	7.3	6.3	0.2	3.9	0.4	0	0.3	0.5	0	0	0.1	0.4	0.1	2	0.4	0.2
37	12.2	13.4	0.9	7.1	0.3	0	0.2	0.6	0	0.2	0.1	0.5	0.1	3.5	0.8	0.4
60	15	17.9	2.7	7.2	0.2	0	0.3	0.5	0	0.4	0.2	0.4	0.2	4.6	0.8	0.5
90	17.1	21.8	6	4.6	0.2	0	0.4	0.3	0.1	0.2	0.4	0.3	0.3	5.6	1.6	0.6
130	18.6	23.8	9	1	0.2	0	0.7	0.2	0.4	0.1	0.6	0	0.5	6.1	3.1	0.7
160	21.2	24.1	10.8	0.2	0.2	0	1	0.2	0.5	0.3	0.6	0	0.8	6.5	4	0.7
180	21.5	24.1	10.7	0	0.2	0	1.1	0.1	0.6	0.2	0.5	0	1	6.9	3.7	0.9
210	21.6	24.2	10.4	0	0.2	0	1.4	0	0.8	0	0.6	0	0	7	3.6	0.9
249	21.8	24.2	9.7	0	0.1	0	1.9	0	0.9	0	0.7	0	1.4	6.8	3.7	0.9

[1] Monomeric. [2] Oligomeric.

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As expected, the carbohydrate and lignin concentrations were increased in the SSL by increasing the H-factor. However, the conversion of hemicelluloses to other products, e.g., furfural, resulted in a decrease in the concentration of xylan/xylose in the SSL. The maximum concentration of xylan/xylose occurred at the H-factor of 160, while the concentrations of furfural, acetic acid, xylonic acid, and methanol continued to increase. Additionally, the concentrations of mono sugars were significantly higher than oligomeric sugars, which would be due to the subsequent hydrolysis of dissolved hemicelluloses.

SSL of NSSC Process

In the production of neutral sulfite semichemical (NSSC) pulp, the chemical pretreatment of wood chips produces the spent liquor that contains a considerable amount of lignocellulosic materials. In one study, the NSSC production was carried out by using hybrid poplar at a temperature of 162-178 ºC, a time of 15-30 min, Na_2SO_3 and Na_2CO_3 dosages of 6.2-11.6%, and 0.8-3.05%, respectively. The SSL contained 2-24% hemicelluloses, 59-69% lignosulfonates, 12-29% acetic acid, and 1.6-2.4% formic acid (*15*). The hemicelluloses possessed two different molecular weights: 8000-19600 and approximately 1000. The lignosulfonate had average molecular weights of 1030 and 235 (*16*). Despite the significant amounts of hemicelluloses and lignosulfonates present in the SSL of the NSSC process, the research on the utilization of this SSL is very limited. In an industrial practice, the SSL is usually treated in the conventional waste water treatment system of the mill, thus the lignocellulosic materials are wasted. Similar to the SSL of sulfite pulping methods, the SSL of the NSSC pulping method may be used in the production of various value added products.

Utilization of SSL via Concentrating

Generally, the low concentration of SSL hampers its utilization in the production of various value-added products. As shown in Figure 1, the conventional evaporation process was used for concentrating the SSL. However, the evaporation process is a costly unit operation with potential fouling problem, which is caused by the precipitation of the inorganic salt (*17*). Considering the high volume of the SSL per tone of production, the incentive for finding a more economical method to increase the concentration of the SSL is high in the industry.

Additionally, there are numerous publications in the literature regarding potential alternative technologies. Ultrafiltration has been reported for increasing the concentration of SSL for many decades. In one study, it was employed to improve the properties of the hardwood and softwood SSLs produced from the ammonium-based dissolving pulp production process. The results showed that the best binding properties for waferboard production were obtained from the treated SSL that contained 50-60% mono sugars. The treatment was conducted using a Diaflo membrane PM-10 (molecular weight cut-off of 10,000), and a DM-5 (nominal weight cut-off of 5,000) (*18*).

To promote the conversion of the dissolved organics in the SSL of the NSSC process to value- added products, the SSL should be initially concentrated. In the literature, ultrafiltration using cellulose acetate membrane was applied for separating reducing sugars of the SSL produced in the NSSC operation, and the reducing sugars concentration was increased from 22-26 % (wt.) to 40-52 % (wt.) (*19*). The rejects of lignosufonates varied from 80-96 % (wt.) with the solid content of 50-80 % (wt.) (*19*).

Production of Various Value-Added Products from SSL

Lignosulfonates

Isolated lignin/lignosulfonate is one of the major products of the biorefinery process for the dissolving pulp production process, as shown in Figure 1. Among the dissolved organics in the SSL of paper-grade sulfite pulp, more than half is lignin (*20*). It was claimed that about 2% of the total dissolved lignin of spent liquor in the world is recovered and marketed (*20*). Approximately, 90% of the market lignin is lignosulfonates obtained from the sulfite process (*20*). Lignosulfonates have a number of commercial usages, including the production of vanillin, animal feed, pellet binders, dispersant for dyes, pesticides, carbon black (*17–21*). The lignin of the SSL is mainly sulfonated, which is a strong organic acid that produces salt with cooking chemicals (*21*). In contract to the dissolved lignin in kraft black liquor, the lignin in the SSL cannot be precipitated by decreasing pH, which makes the separation process more challenging (*20*, *22*, *23*). Ion exchange, dialysis, electrodialysis were used in a laboratory scale for separating lignosulfonates (*24*).

The recovery of lignosulfonates from the SSL of paper-grade pulp via ultrafiltration has been extensively studied (*25–27*). There are varieties of porous membranes available for concentrating the SSL to a desired level (*28*).

The reverse osmosis can also be employed for separating a solute/solvent of a similar molecular size. However, it needs a relatively tight membrane, and salt rejection may impair the separation (*21*). In one study, a combination of ultrafilteration and reverse osmosis was used for separating the lignosulfonate and sugars from the softwood SSL (*21*). Figure 3 shows the separation of lignocellulosic materials from the softwood SSL using a membrane (no detailed information was given on the membrane used).

In this process, the concentration of lignosulfonates increased from 10% to 30% (wt.) by ultrafiltering. By applying reverse osmosis on the hemicelluloses stream, the concentration of hemicelluloses increased from 10% to 20% (wt.). The water produced is clean and it can be recycled to the pulp production unit of the mill. Since the water content decreases dramatically via ultrafiltration, it may be economical to use evaporation for further concentrating of the lignocellulosic stream for the production of value added materials (*21*).

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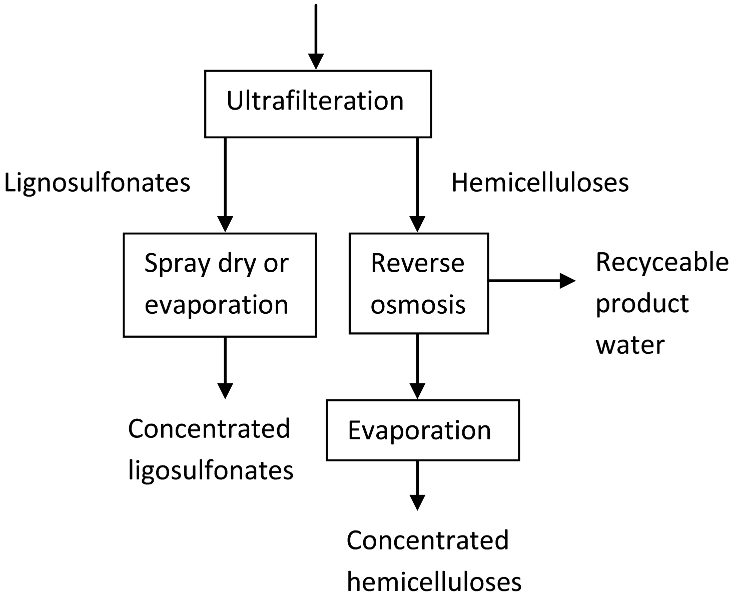
Ultrafilteration
Lignosulfonates
Hemicelluloses
Spray dry or evaporation
Reverse osmosis
Recyceable product water
Concentrated ligosulfonates
Evaporation
Concentrated hemicelluloses

Figure 3. Process flow diagram of lignin and hemicelluloses recovery of calcium-based SSL using ultrafilteration and reverse osmosis. (Reproduced with permission from reference (21). Copyright 1975 Tappi).

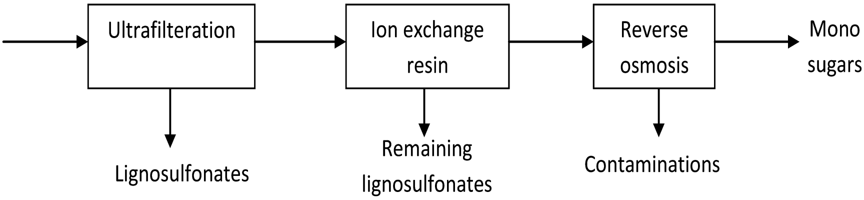
Ultrafilteration
Ion exchange resin
Reverse osmosis
Mono sugars
Lignosulfonates
Remaining lignosulfonates
Contaminations

Figure 4. A combination of ultrafilteration and reverse osmosis system applied on the SSL of magnesium-based sulfite pulping process for separating lignosulfonates and sugars. (Reproduced with permission from reference (29). Copyright 2009 Elsevier).

Another configuration of ultrafiltration and reverse osmosis was introduced in the literature for separating the dissolved sugars and lignosufonates of the paper-grade SSL (*29*). Figure 4 shows the process applied for separating lignosulfonates and sugars of the SSL of the magnesium-based sulfite pulping process. The most promising ultrafiltration system consisted of a Microdyn-Nadir UP010 membrane that could separate the high MW lignosulfonates from the low MW dissolved organic in the SSL. However, ultrafiltration could not separate the hemicelluloses from lignin, due to the overlap of the molecular weight of lignin and hemicelluloses dissolved in spent sulfite liquor (*29*). To separate the rest of lignosulfonates from the SSL, an ion exchange resin was used, which was followed by a reverse osmosis system using Alfa Laval RO99 with glucose and xylose retentions of 96% and 93%, respectively (*29*).

The isolation of lignosulfonates was industrially practiced using ultrafiltration via polysulphone, cellulose acetate, fluropolymer (*30*, *31*). The liquid bulk membrane using trioctylamine as a carrier, and dichloroethane as a solvent was also proposed for separating lignosulfonates from solutions. In this case, the maximum separation (90-98%) is obtained at feed phase pH of 2 and a concentration of 4% v/v (*32*). In another study, the addition of surfactants (polyethylene glycol) changed the liquid membrane to emulsion membrane. The maximum removal (91%) of lignosulfonates was achieved under the conditions of 5% surfactant concentration (w/v), strip phase concentration of 0.4 M NaOH, and a phase ratio of 1:1. The extraction of lignosulfonates increased with increasing the concentrations of strip phase and carrier (*33*). No results are available on the efficiency of liquid or emulsion membrane in separating lignosulfonate from the SSL. However, the complex chemical composition of the SSL limits the potential use of this process in industry.

An amine extraction has also been proposed in a laboratory scale for separating lignosulfonates of the SSL produced in dissolving pulp production. In this process, lignosulfonates are transferred to lignosulfonic acid-amine adducts, which are insoluble in water. The adducts can be subsequently isolated based on the liquid-liquid extraction (*34*). Different materials have been proposed for extracting lignosulfonates from various SSLs using this system such as: long chain aliphatic amine, tri-n-hexyl-amine, and poly ethyleneimine (*28*, *35–37*). The main problem of amine extraction is the required complete removal of the amine, formation of NaCl during re-extraction, foam, and emulsion problems, as well as the time- consuming separation procedure (*28*). Additionally, the high variability in molar mass and degree of sulfonation complicates the extraction process. The high consumption of organic solvent, a limited number of by-products, and a high polydispersity of lignin obtained limit the potential of this process for industrial scale operations (*28*).

The Pekilo process was also proposed for separating lignosulfonate from the SSL, which is a combination of fermentation and ultrafiltration. In this system, the SSL is first stripped, and fermented using a micro fungus *Paecilomyces varioti* along with the other required chemicals (Figure 5). The fungus consumes hexoses, pentoses and acetic acid to produce cell mass containing 55-60% of protein, CO_2 and water. Generally, 570 g of Pekilo protein is produced per 1 kg of monosaccharide consumption. In one study, the softwood SSL was used in the Pekilo process (no information was given on the fermentation conditions) (*38*). The SSL contained 55% lignosulfonates, 27% monosaccharides, and 3% acetic acid. After the fermentation, it contained 77% lignosulfonates, 1% monosaccharides, and a traceable amount of acetic acid. Ultrafiltration produced lignosulfonates with a purity of more than 90%. It was proposed that the filtrate be recycled to a reservoir located prior to ultrafiltration (stream 1) in the process. The advantages would be to adjust the concentration of the cake (lignosulfonates) and to improve the purify the lignosulfonate product. The filtrates may contain low molecular weights lignosulfonates and mono saccharides. The separated lignosulfonates would have a relatively high molecular weight. However, as

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it may contain some monosacharides and acetic acid, we propose stream 2 for further improving the efficiency of monosaccharide utilization in the process. Major disadvantages of this process are the slow fermentation process and high maintenance/operating cost of the ultrafiltration.

Vanillin

The production of vanillin from the lignosulfonates of the SSL was commercialized (*39*), however, was stopped after the World War II due to the redcued cost of alternative sources of vanillin. Vanillin, acetovanillone, guaiacol, and *p*-hydroxybenzaldehyde are low molecular weight aromatic compounds produced from oxidation of lignin in the SSL. Figure 6 shows four different pathways for producing vanillin from the SSL. As can be seen, the SSL is oxidized prior to further processing via these methods. The oxidation can be conducted under an alkaline environment by heating under air or oxygen at a temperature of 160 ºC. During oxidation, some other products, e.g., oxidized lignin, acetovanillone, dehydrodivanillin, guaiacol, p-hydroxybenzenaldehyde, and aromatic acids can also form. The oxidized SSL can be evaporated to concentrate the vanillin solution (stream 1), but the high solid content of the oxidized SSL hinders the evaporation process. The oxidized SSL can be acidified, subsequently; sodium vanillate is extracted using solvents such as toluene (*39*) (stream 2). Alternatively, the oxidized SSL can be extracted directly using n-butyl alcohol or isopropyl alcohol to isolate sodium vanillate (*40*, *41*) (stream 3). However, the limited solubility of sodium vanillate hampers the practicality of this method. Another option is to further purify the oxidized SSL via using a cationic exchange resin, which adsorb sodium vanillate (stream 4). The eluent of sodium vanillate from the column is performed after each adsorption using either water or sodium carbonate solution (*41*). In this way, the column maintains its sodium form and is immediately ready for a new adsorption batch without the demand for a regeneration step. In one study on the SSL of spruce species using a cationic exchange resin in its sodium form (Dowex-50 W, X-2, 200-400 mesh), the following advantages were reported via following stream 4 (*41*): about 80% of lignin, sodium and dry matter were separated from vanillin-containing fraction; the quantity of acid needed to neutralize the vanillin fraction was small in reference to the quantity needed for the oxidized SSL; no lignin precipitation occurred, which facilitated the extraction process of vanillin; equipment cost was low with no pressurized equipment needed.

Alternatively, the SSL can be initially ultrafiltered, whereby lignosulfonates are isolated from the SSL. Vanillin is then produced from the lignosulfonates by oxidation. The disadvantage of this system is the large amount of acid required for the neutralization prior to the extraction of vanillin, and ineffective extraction of sodium vanillate. The neutralization causes the lignin precipitation, and the extraction takes place from a large volume of a solution having a high solid content, but a low vanillin concentration (*41*).

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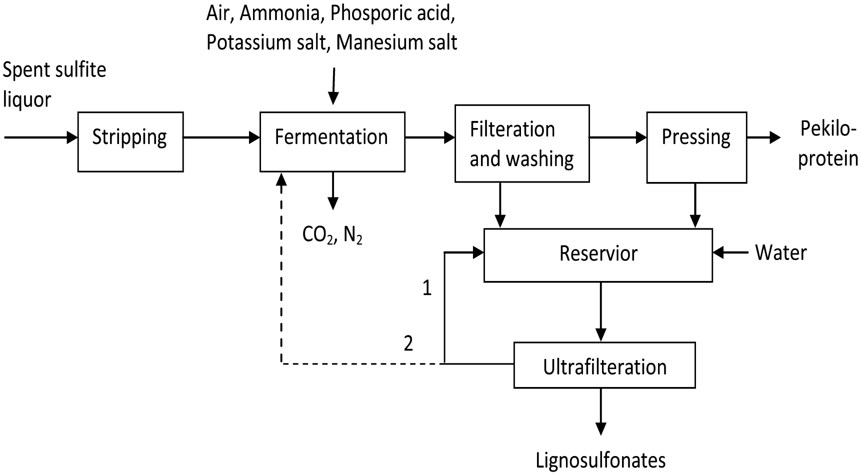
Air, Ammonia, Phosporic acid,
Potassium salt, Manesium salt
Spent sulfite liquor
Stripping
Fermentation
Filteration and washing
Pressing
Pekilo-protein
CO_2, N_2
Reservior
Water
1
2
Ultrafilteration
Lignosulfonates

Figure 5. Pekilo process for recovering of lignosulfonates from the SSL. (Reproduced with permission from reference (38). Copyright 1979 PAPTAC).

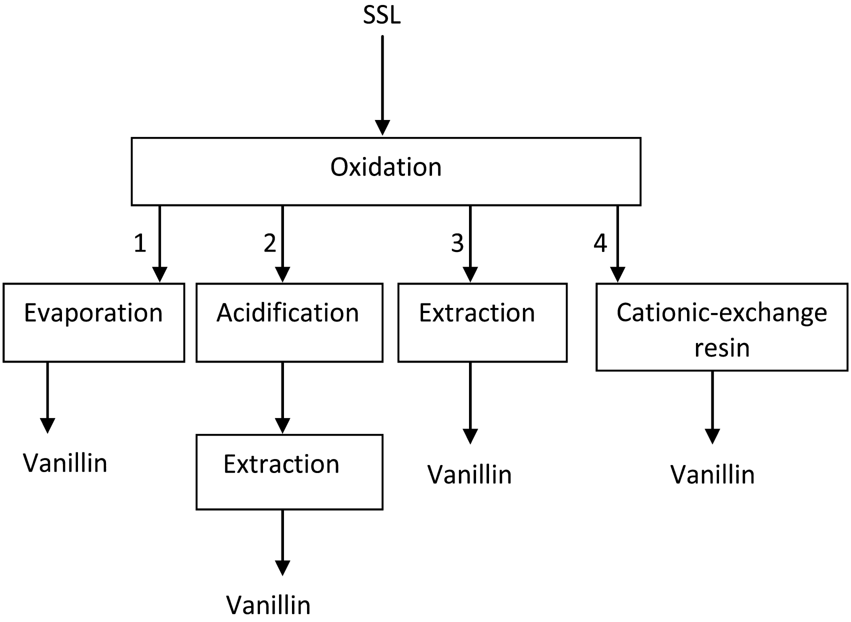
SSL
Oxidation
1
2
3
4
Evaporation
Acidification
Extraction
Cationic-exchange resin
Vanillin
Extraction
Vanillin
Vanillin
Vanillin

Figure 6. Proposed processes for the production of vanillin from SSL. (Reproduced with permission from reference (41). Copyright 1986 ACS).

Xylitol

Xylitol has a commercial use as sweeteners and a possible usage in adhesives (*42*, *43*). The xylose present in the SSL can be converted to xylitol via hydrogenation or fermentation using some microorganisms.

Hydrogenation is a commercial method to produce xylitol from xylose (*44*). In this method, xylose is converted to xylitol in the presence of Raney nickel, as a catalyst, under alcohol aqueous solution (*44–47*). The hydrogenation of xylose also produces small amount of xylulose as a by-product. The xylulose can be hydrogenated to xylitol and arabinitol. Under extreme conditions of high temperature and alkali concentration, furfural and xylonic acid are also produced as by-products. The main by-products of this process are xylulose and arabinitol. The formation of such products can be suppressed by low temperature and high hydrogen pressures (*45*).

Production of xylitol from the SSL via fermentation is intensely studied in various laboratories. Since the hardwood SSL contains a significant amount of xylose, it is a desirable raw material for xylitol production (see Tables I and II). In the literature, certain organisms such as *Candida boidinii* (*48*), *Candida guilliermondii* (*49*), *Candida tropicalis* (*50*), *Candida parapsilosis* (*51*), and *Debaryomyces hansenii* (*52*) were studied to metabolize xylose to xylitol through xylose reductase (XR) catalysis. Similar to the hydrogenation process, the produced xylitol can also be re-oxidized to xylulose by xylitol dehydrogenase. In one study, *Pichis stipitis* FPL-YS30 was applied to diluted (15% wt.) ammonia-based SSL of pine, xylose, and glucose samples for converting the sugars to xylitol. Initially, different yeast peptone sugar media were inoculated with pure cultures from inocula grown in different medial of sugars or SSL to obtain a concentration of 0.2 g DCW/l. Then, the fermentation was conducted at 30 ºC at 200 rpm for different time intervals. Figure 7 shows the cell production, pH changes, and xylose concentration of SSL at different fermentation time intervals.

It was evident that the cell concentration was increased, while the pH and xylose concentration were decreased as the fermentation time elapsed. Also, the cell concentration was lower in the pre-cultivated SSL sample than other samples, which implies that the cell could grow more in pre-cultivated xylose or glucose than in that of the SSL. However, the xylose concentration and pH changes were similar. Figure 8 shows the xylitol, arabitol, and glycerol concentrations at different fermentation time intervals of the above system.

It was observed that the xylitol and glycerol concentrations were increased as the fermentation time passed, while the arabitol concentration reached a plateau, regardless of the pre-cultivation medium. Also, the concentration of xylitol, arabitol, and glycerol were lower for the pre-cultivated SSL samples than for other samples (*43*). The lower xylitol production is attributed to the presence of inhibitors in the SSL. The inhibitors many include: 1) metal and mineral presence in the SSL, 2) products derived from the hydrolysis of hemicelluloses, e.g., acetic acid, furfural and hydroxymethylfurfural (HMF), 3) products from the lignin and extractive degradations (*43*, *52*). To investigate the effect of the inhibitors during fermentation using *Candida guilliermondii*, the xylitol

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production was followed under different medium concentrations of furfural, vanillin and syringaldehyde on a laboratory scale (*42*). Generally, increasing their concentrations increased the severity of inhibition in terms of a) the reduced specific growth rate, b) increased lag time, and c) reduced xylitol production rate. It was reported that the xylitol production was the highest at a very high xylose concentration (80 g/l) (*53*, *54*). However, concentrating the SSL based on the conventional evaporation technology can also increase the concentrations of the above-mentioned inhibitors present in SSL. Alternatively, overliming, adsorption on activated carbon, removal with ion-exchange resins and vacuum evaporation were proposed to reduce the concentration of inhibitors for xylitol fermentation in the literature (*55–57*).

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Xylanase

The high concentration of xylose in hardwood SSL of dissolving pulp production promotes the xylanase production. In one study, the concentrated SSL was collected from the third evaporator of acid-based sulfite pulping process and used as a raw material for the production of xylanase with various *Aspergillus oryzae*, *Aspergillus phoenicis*, *Aspergillus foetidus* ATCC 14916, *Aspergillus niger* ATCC 10864, *Gliocladium viride* CBS 658.70 (*58*). It was evident that *A. oryzae* NRRL 3485 and *A. phoenicis* ATCC 13157 were more productive than others in producing xylanase. The maximum of 200 U/ml xylanase was produced, while the biomass production was about 10 g/l after 100 h of the treatment with *A. oryzae* NRRL 3485 or *A. phoenicis* ATCC 13157 at a pH of 6 at 30 ºC on the 10-fold diluted SSL (*58*). The optimum pHs of the above system for the enzyme preparations of *A. oryzae* or *A. phoenicis* were 6.5 and 5.0, and the optimal temperatures were 65 ºC and 50 ºC, respectively. Similar to xylitol production, the xylanase production was also negatively affected by the presence of inhibitors, such as acetic acid, in the SSL (*58*).

Ethanol

As shown in Tables I, II, and III the SSL of dissolving pulp- or paper-grade sulfite pulping process usually contains a significant amount of sugars that can be utilized for ethanol production (*59*). The ethanol production from the SSL of sulfite-based dissolving pulp production process has been industrially practiced for a long time (*10*, *11*). In this process, the SSL is concentrated via evaporation, and subsequently the hemicelluloses/sugars present in the concentrated SSL are utilized for ethanol production via fermenting by *S. cerevisiae*. The commercial ethanol production capacity of an Eastern Canadian biorefinery unit via this process was 18 million liter/year in 2006 (*10*). The details of this process were described above and the process configuration was presented in Figure 1.

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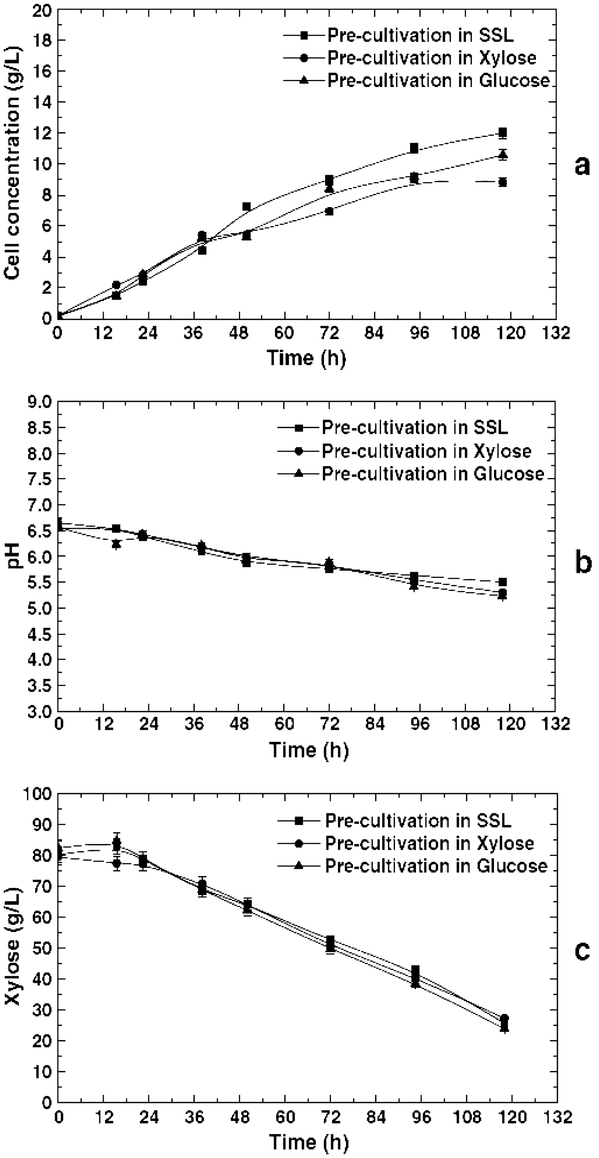

Figure 7. a) Cell production (g/l); b) pH; c) xylose concentration (g/l) during fermentation by P. stipitis D-xylulokinase mutant. (Reproduced with permission from reference (43). Copyright 2008 Springer).

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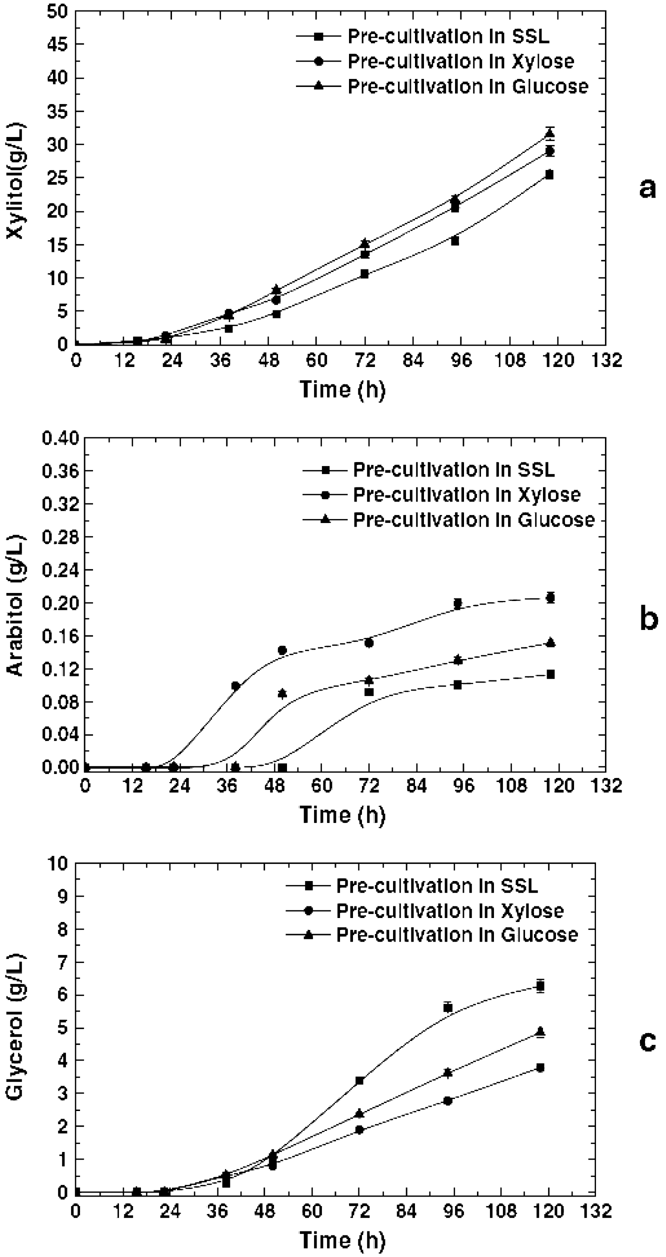

Figure 8. a) Xylitol; b) arabitol; c) glycerol production during fermentation by P. stipitis D-xylulokinase mutant. (Reproduced with permission from reference (43). Copyright 2008 Springer).

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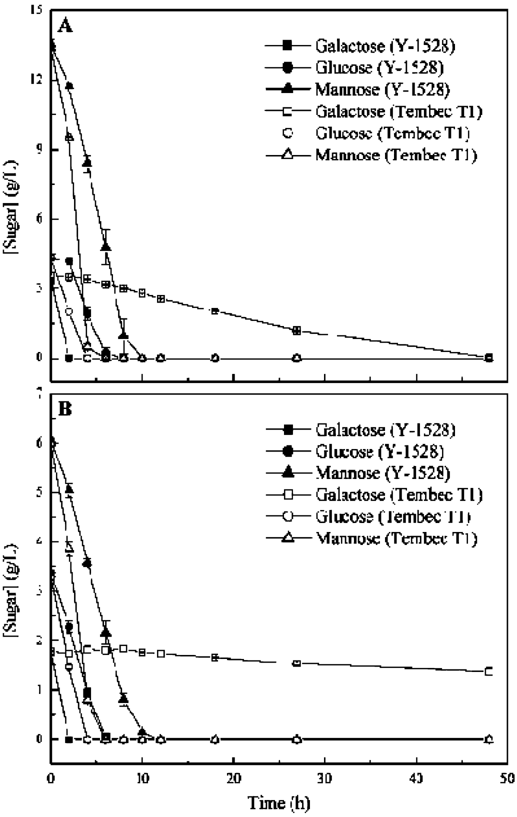

Figure 9. Consumption of hexose sugars present in a) softwood SSL; b) hardwood SSL by S. cerevisiae Y-1528 or Tembec T1, following growth on glucose. (Reproduced with permission from reference (61). Copyright 2004 Springer).

Additionally, several microorganisms were studied for the production of ethanol from the SSL of dissolving pulp production process in laboratory scales (*4*, *11*, *60*, *61*). The SSL contains three hexose sugars, i.e., galactose, glucose and mannose, and two pentose sugars, xylose and arabinose. The consumption rates of these sugars are different for various microorganisms. It was reported that the fermentation rate of xylose is 3-12 times lower than that of glucose by *S. cerevisiae* (*4*). It is therefore important in the manufacture of ethanol to give preference to softwood SSL due to higher concentration of hexose sugars versus pentose sugars.

In the literature, *S. cerevisiae* was applied for fermenting the SSL of hardwood and softwood at 30 °C and 125 rpm for 48 h in a laboratory scale (*61*). Figure 9 shows the consumption of hexose sugars of the softwood and hardwood SSL for the production of ethanol using *S. cerevisiae* Y-1528 or Tembec T1 (*61*).

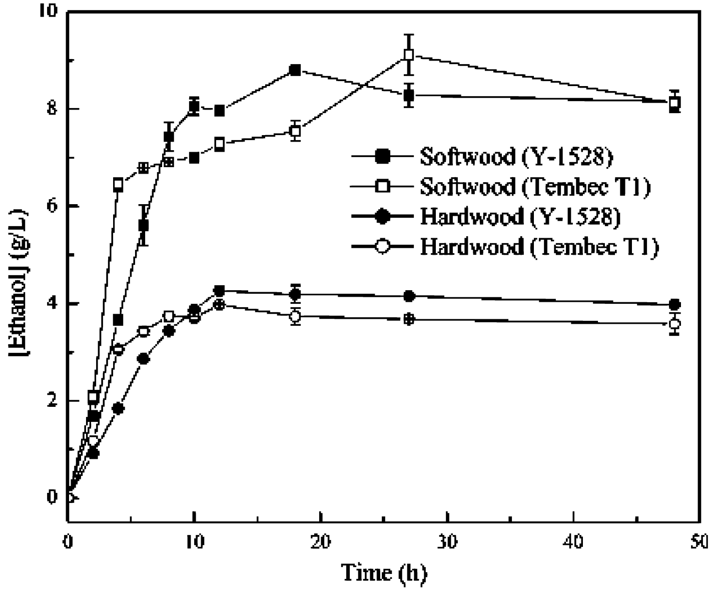

Figure 10. Ethanol production during softwood and hardwood SSL fermentation by S. cerevisiae Y-1528 and S. cerevisiae Tembec T1. (Reproduced with permission from reference (61). Copyright 2004 Springer).

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As can be seen, galactose was consumed faster than the other sugars using Y-1528, and the consumption of sugars reduced remarkably in 30 h. However, the consumption rate of galactose by Tembec T1 was very low. Figure 10 shows the ethanol production using the softwood and hardwood SSL by *S. cerevisiae* Y-1528 or Tembec T1.

Evidently, the ethanol production reached the maximum in 10 h, and the ethanol production of the softwood SSL was twice as much as that of the hardwood SSL. This is because there are more hexoses in the softwood SSL than in the hardwood SSL, and the amount of inhibitors was higher in the hardwood SSL. The higher ethanol production using Y-1528 implies that Y-1528 is more tolerable than Tembec T1 to the inhibitors present in the SSL (*61*).

The main challenges for ethanol production from the SSL are the low nutrient concentration, the presence of inhibitors, and the large proportion of xylose. The yeasts that are capable of fermenting pentoses are also limited (*60*). In the following subsections, the procedures introduced for overcoming the above-mentioned challenges are described.

Removal of Inhibitors

Natural occurring and process-induced compounds may retard the ethanol production, which complicates the fermentation process (*4*, *12*). The inhibitors may include phenols, acetic acid, furfural, pulping chemicals (mainly metal ions) and lignosulfonates. These inhibitors can be chemically or biologically removed from the SSL.

Boiling and overliming have been proposed as methods to reduce the concentration of inhibitors in the literature (*12*, *60*). Boiling was proposed to reduce the concentration of volatile components, e.g., furfural, and overliming was proposed to create some insoluble inorganic salts, which adsorb inhibitors (*62*). Figure 11 shows the procedure applied to reduce the inhibitors of the hardwood SSL based on boiling and overliming by Nigam (*62*).

As can be seen, the hardwood SSL was first boiled at 100 ºC for 25 min, and then overlimed to increase the pH of the SSL to 10. In this step, the presence of calcium oxide and sulfate ions in the liquor resulted in the formation of gypsum (*62*). The gypsum formation facilitated the adsorption and removal of lignosulfonates from the SSL. The lignosulfonates-adsorbed gypsum was removed in the filtration step. Subsequently, the pH was adjusted to 6.5, and the concentration of this SSL was increased via vacuum filtering at 25ºC to have a xylose concentration of 4-5% w/v. The ethanol fermentation was conducted on the treated SSL using *Pichia stipitis* or *Pichia stipitis* (A) at 30 ºC, and pH of 6.5. The details about the adoption procedure of *Pichia stipitis* were comprehensively described by Nigam (*62*). Figure 12 shows the ethanol and sugar concentrations versus the time of fermentation for the treated SSL.

As shown, the ethanol production was evident, while the sugar concentration was remarkably reduced using *P. stipitis* (A) on the treated SSL of the above-mentioned system. Also, the ethanol production and sugar consumption were limited for the untreated SSL, which was ascribed to the high concentration of inhibitors (*62*). In this system, the maximum ethanol productivity was 0.44 g/l/h (*62*).

In another study on ammonia-based hardwood and softwood SSL, *Escherichia coli* B (ATCC 11303 pLOI297) was applied in anaerobic batch fermentation process at a pH of 7 and 30 ºC (*11*). The maximum conversion efficiency of sugars to ethanol was 67-84% for hardwood SSL and 53-76% for softwood SSL. The overliming improved the productivity of the ethanol production from hardwood SSL two- to seven-fold to a maximum of 0.42 g/l/h with the conversion efficiency of 92% (*11*).

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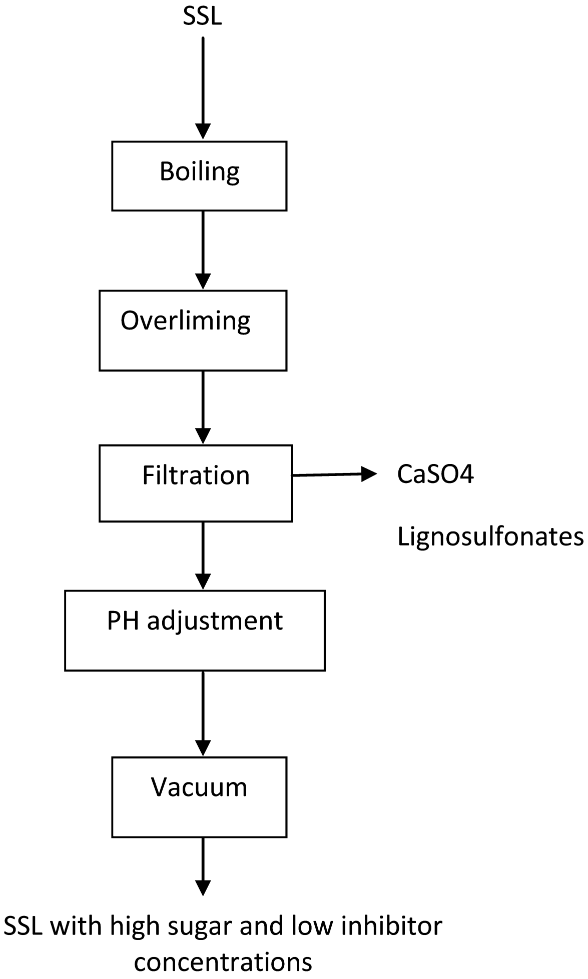
SSL
Boiling
Overliming
Filtration
CaSO4
Lignosulfonates
PH adjustment
Vacuum
SSL with high sugar and low inhibitor concentrations

Figure 11. Process configuration of the SSL for reducing the concentration of inhibitors for ethanol production. (Reproduced with permission from reference (62). Copyright 2001 Springer).

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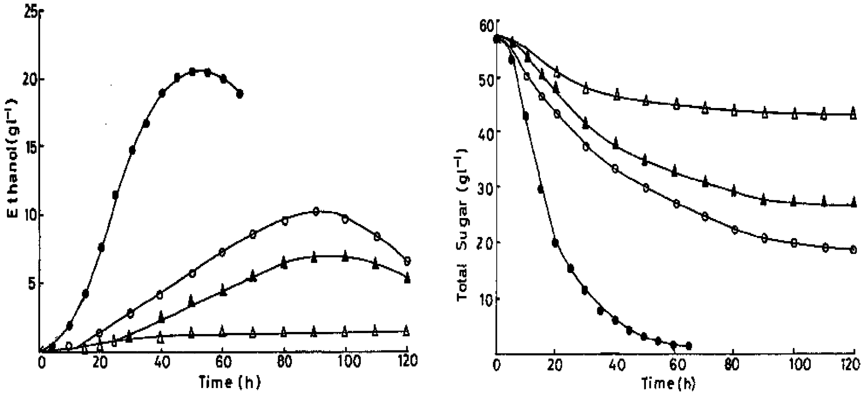

Figure 12. Ethanol production and sugar consumption by P. stripitis (triangle) untreated SSL; (filled triangle) untreated SSL by P. stripitis (A); (circle) treated SSL by P. stripitis; (filled circle) treated SSL by P. stripitis (A). (Reproduced with permission from reference (62). Copyright 2001 Springer).

The application of ion exchange resins was reported to be an efficient separation method for separating neutral sugars from the inhibitors present in the hardwood SSL of paper-grade sulfite pulping (*63*).

Alternatively, the inhibitors of the SSL can be removed by employing the concept of adsorption/flocculation phenomenon. In this process, commercial adsorbents, e.g., activated carbons, fillers, e.g., calcium carbonate or lime, or ion exchange resins may be appropriate alternatives. The research on the performance of these adsorbents in removing the inhibitors of various SSL of sulfite-based or pre-hydrolysis liquor of kraft-based dissolving pulp production process is on-going. The details of the process concepts involved in utilizing these adsorbents are described in Chapter 18.

Alternatively, the inhibitors can be removed from the SSL media via a biological treatment. For example, a mutant yeast, *S. cerevisiae* YGSCD 308.3, was applied to reduce the acetic acid content of the ammonia-based hardwood SSL in the literature (*64*). This yeast grows on acetic acid, but not on xylose, glucose, mannose and fructose. The fermentation was conducted at a temperature of 30 ºC, and 300 rpm for 24 h in a laboratory scale, which resulted in 73% of the theoretical ethanol production in 24 h (*64*). The acidic environment was preferred for the acetic acid removal in such a system. However, the mutant converted a small fraction of xylose to xylitol.

Another alternative to increase the ethanol productivity is to employ a pentose-fermenting microorganism. In one study on a calcium-based SSL, *Rhizopus oryzae* CCUG 28958 (a filamentous fungus) was applied for fermentation. *R. oryzae* was originally introduced to produce lactic acid, but it was also capable of producing ethanol via consuming hexoses and pentoses present in the SSL (*6*). The incubation under the conditions of 170 rpm, and 30 ºC for 5-14 days resulted in the maximum ethanol, lactic acid, and glycerol yields of 0.37, 0.3 and 0.09 g/g. In general, there was a direct competition between lactic acid and ethanol among the metabolites. Poor medium compositions and cultivation resulted in a higher yield of lactic acid, while a rich media resulted in a higher biomass and ethanol yields (*6*). In another study on a magnesium-based SSL, all of the acetic acid was removed by a biological treatment with *S. cereviasiae* 4072 at 28 ºC for 3 days. However, the presence of polyphenolic compounds inhibited the fermentation via *P.stipitis* at 60 ºC (*6*). In another study on a calcium based-SSL, the fermentation was followed using a combination of *S. cereviasiae* and fungi (*65*). Using *S. cereviasiae* at 27 ºC and 0.8 g/l concentration resulted in 8.64 g/l ethanol production. However, when *Chalara parvispora* and *Trametes versicolor* fungi (1 g/l concentration) were applied along with S. cereviasiae (0.8 g/l concentration), the ethanol concentration was increased to 24.61 g/l (*65*).

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Enriching the SSL

Generally, the large xylose content of SSL, especially for hardwood SSL, has negative effect on the ethanol production, which is due to the fact that the available yeast used in industry are capable of fermenting glucose, but not xylose. If the SSL contains a high amount of xylose, the ethanol production rate and capacity are usually low, as described earlier. One method to overcome this difficulty is to increase the yeast concentration for improving the ethanol production (*12*). Alternatively, the shortcoming of hardwood SSL, which is low in the hexose concentration, could be improved by the addition of hexoses, which in turn improved the yeast concentration. The increased concentration could improve the fermentation rate (*12*). In another study, the reject knots from the sulfite pulping were used as a hexose source to fortify the SSL (*66*). In this study, the knots were refined at 2% consistency and then the refined knots were hydrolyzed with enzymes in a laboratory scale.

Modifying Yeast for Better Performance

Many studies are available in the literature on the development of better yeast for the ethanol production from the SSL. In one study, the SSL-adapted yeast strain T2 and a genetically-modified *S. cerevisiae* 259ST were applied to produce ethanol from a hardwood SSL (*4*). It was observed that the ethanol production ranged from 0.32 to 0.42 g/g for 259ST, while it was 0.15-0.32 g/g for non-xylose fermenting strain T2 at a pH of 6 (*4*). Also, the minimal amount of xylitol (< 1g/l) was produced and glycerol yield was 0.12 g/g (*4*). The genetically modified *S. cerevisiae* 259ST was significantly more effective than the unmodified *S. cerevisiae* in producing ethanol (*12*). Although the adoption improved the efficiency of *P.stipitis* tolerance to wood hydrolysates, the process is time consuming and labor intensive.

Alternatively, a random mutagenesis was applied by using UV followed by a selection to obtain *P. stipitis* with improved tolerance to inhibitors of the hardwood SSL (*5*). The UV mutagenesis was conducted at a distance of 40 cm for 20 s to achieve 50% survival rate with a subsequent incubation at 60 ºC for 24 h on *P. stipitis*. Subsequently, they were incubated on the hardwood SSL (pH 5.5) at 28 ºC for 5-10 days. The processed yeast retained the ability to utilize and ferment four major sugars (glucose, galactose, xylose and mannose), but not arabinose. The modification improved the ability of mutant to utilize glucose-xylose mixture (*5*).

Biogas

Biogas could be a value-added by-product of the SSL, and hence become a part of the integrated forest biorefinery unit for the sulfite process. Alternatively, it could be produced from the effluent of pulping or bleaching stages. In an ammounium-based sulfite pulping process, the biogas production from the bleaching effluent is practiced by employing an anaerobic digester (*10*). The utilization of this process showed that the chemical oxygen demand (COD) level of the wastewater could be reduced by 65% (*10*). In this case, the amount of methane produced was sufficient to supply the heat demand of the pulp dryer of the mill. Alternatively, it can be sold as a product in the open market.

Ethanol Production via SPORL Process

Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) has been developed by the U.S. Forest Service, Forest Products Laboratory and the University of Wisconsin-Madison as a promising biorefinery process (*67*, *68*). The differences of sulfite and the SPORL processes include: 1) the main product of sulfite process is pulp, while that of the SPORL process is ethanol; 2) depending on the downstream usage of the pulp, the quality of pulp produced in the sulfite process is of great importance, while the sugar composition and concentration (yield and/or recovery) are important in the SPORL process; 3) the sulfite process targets on removing lignin, and probably hemicelluloses, from the cellulose substrate, while the SPORL process targets on producing monomeric sugars from hemicelluloses and cellulose to ferment to ethanol.

To achieve the above targets, the sulfite and SPORL processes have different process criteria: the sulfite pulping process has a careful and extended delignification (cooking) process, which is operated at a relatively low temperature. However, the SPORL process has a short chemical pretreatment at a high temperature to remove recalcitrance of the substrate without a significant delignification, and a disk refiner to increase the surface area, which is necessary for the sugar dissolution in the latter stages for fermentation.

The potential products of the SPORL process are hemicellulosic sugars, lignosulfonate and ethanol (*8*). Figure 13 shows the process diagram of the SPORL process. As can be seen, the wood chips are pretreated with bisulfite and/or sulfuric acid at a temperature of 160-190 ºC, a pH of 2-5, and L/W ratio of 2-3 for 10-30 min. The bisulfite charge is 1-3% and 6-9% for hardwood and softwood species, respectively, and acid ranges between 1 to 2% (*7*). The solid substrates of the chemical treatment are then fibrilized using a mechanical disk refiner. The chemical pretreatment has a direct effect on the refining stage of

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the SPORL process. By decreasing the solid content of the treated materials sent to the disk refiner and increasing disk plate gap, the energy requirement of the refiners is significantly reduced (*8*). Subsequently, the solid substrate is enzymatically hydrolyzed, fermented, and distilled to produce ethanol (*8*). Softwoods species have usually poor digestability in enzymatic saccharification (*67*). The SPORL process is particularly effective in improving the enzymatic saccharification efficiency of softwoods. The sugars in the spent liquor from the pretreatment SSL of the SPORL can also be fermented for increasing the ethanol production similar to the Pekilo process (*8*, *69*).

The SPORL process can be integrated with a steam explosion pretreatment by using sulfite and acid as catalysts. It was reported that the SSL of the SPORL process is easier to ferment compared with the SSL of the sulfite process, due to its high hemicelluloses content (*7*). The pretreatment of the wood chips under various conditions described above can result in nearly complete removal of hemicelluloses. The removals of hemicelluloses of various wood species under different conditions are listed in Table IV. Interestingly, except for eucalyptus and pine species, the xylan removal was nearly complete for other species. The mannose removals were 93% and 61.6% for spruce and pine, respectively, but no mannose was removed from the hardwoods. The glucan removal found to be minimal under the conditions investigated.

The SPORL process is achieved by a combined effect of the dissolution of hemicelluloses under a low pH, partial depolymerization of cellulose, partial delignification, partial sulfonation of lignin, and increasing the surface area of woody materials by mechanical refining. By controlling the pH, temperature, and sulfite dosage of the SPORL process, the condensation of lignin is prevented (*68*).

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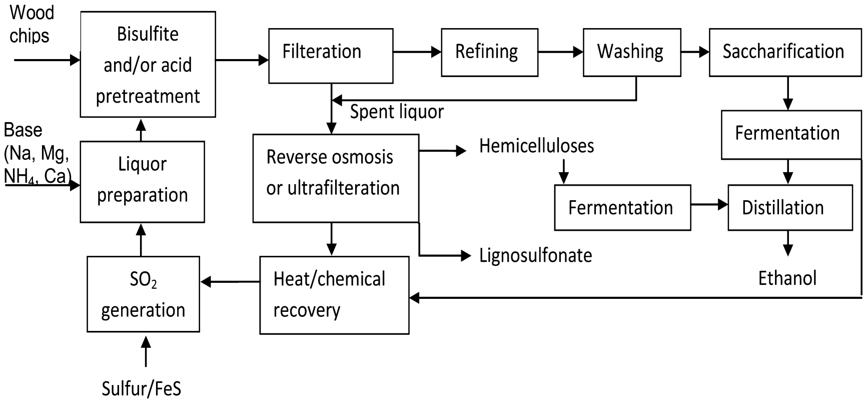
Wood chips
Bisulfite and/or acid pretreatment
Filteration
Refining
Washing
Saccharification
Spent liquor
Fermentation
Base (Na, Mg, NH4, Ca)
Liquor preparation
Reverse osmosis or ultrafilteration
Hemicelluloses
Fermentation
Distillation
Lignosulfonate
SO2 generation
Heat/chemical recovery
Ethanol
Sulfur/FeS

Figure 13. Process flow diagram of the SPORL or dilute acid treatment. (Reproduced with permission from reference (8). Copyright 2010 Elsevier).

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Table IV. The conditions of SPORL process and resulting hemicelluloses removal (%) for various wood species. (Reproduced with permission from references (*8*, *67*, *68*). Copyrights 2009, 2010 Elsevier and 2009 AICHE)

Wood species	*Temperature, ℃*	*Time, min*	*Bisulfite, %*	*Sulfuric acid, %*	*Glucan, %*	*Mannan, %*	*Xylan, %*	*References*
aspen	180	30	4	2.76	17.9	n/a	98.2a	(*68*)
Aspen	180	30	4	0.92	10.5	n/a	91.3a	(*68*)
Eucalyptus	180	30	6	1.84	2.1	n/a	77.5a	(*68*)
Spruce	180	30	9	3.68	11.2	93	98.2	(*67*)
Pine	180	30	8	2.21	9.4	61.6	45.9	(*8*)

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Table V. Chemical analysis of original spruce, pretreated spruce substrate and its SSL for dilute acid and SPORL processes. (Reproduced with permission from reference (*70*). Copyright 2010 Elsevier)

	Arabionse	*Galactose*	*Glucose*	*Xylose*	*Mannose*	*Acid-soluble lignin*	*Acid-insoluble lignin*
Original spruce, %	1.2	2.6	46.7	5.5	10.8	29	1.1
Dilute acid substrate, %	ND	ND	51.9	ND	ND	48.5	0.8
SPORL substrate, %	ND	ND	66.6	ND[1]	1.2	32.9	1.2
Dilute acid hydrolysate, g/l	0.2	0.8	6	0.4	1.7	NA[2]	4.8
SPORL hydrolysate, g/l	0.8	2.6	5.7	4.3	9	NA	16.6

[1] ND-not detected. [2] NA-not applicable.

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Table VI. The concentration of sugars in hydrolysate and substrate of pine species (kg/ton wood). (Reproduced with permission from reference (*8*). Copyright 2010 Elsevier)

				Substrate						*Hydrolysate*					
Pretreatment	*Initial pH*	*Final pH*	*Acid charge, %*	*Bisulfate charge, %*	*Glucan*	*Xylan*	*Mannan*	*K lignin*	*Total solids*	*Glucose as glucan*	*Xylose as xylan*	*Mannose as mannan*	*Lignin*	*Yield*	*Total yield*
Dilute acid	1.1	1.5	2.21	0	355.8	1.1	0.5	334.3	627.5	39.4	ND	4.4	ND	43.8	781.5
SPROL	4.2	2.5	0	8	412.4	16.1	7.7	226.3	662.5	11.2	23.5	42.6	43.8	121.1	783.6
SPORL	1.9	1.5	2.21	8	383.5	3.2	2.5	254.3	643.5	40.1	31.8	67.7	15.8	155.4	798.9

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Ethanol can be produced by pretreating of wood chips with sulfuric acid, and subsequent mechanical pulping (refining) of the pretreated wood chips. This process is called a dilute acid process. However, a high temperature and a very low pH of the process impart serious equipment corrosion problems (*70*). The chemical analyses of original spruce, pretreated spruce substrate and its spent liquor from the dilute acid and the SPORL processes are listed in Table Table V. The acid pretreatment was conducted under 180 ºC for 30 min with a sulfuric acid loading of 5% on wood and liquor/wood ratio of 5, while the SPORL process was supplemented with 9% sodium sulfite.

As can be seen, almost all of the hemicelluloses dissolved during the dilute acid pretreatment, leaving the lignin and cellulose in the substrate. However, the condensation of lignin happened, which increased the lignin concentration in substrate. In contrast, the SPORL pretreatment retained more glucose, but less lignin, in the substrate. The difference between these two pretreatments was attributed to the sulfite addition. In fact, the sulfite addition increased the pH of the pretreatment from 1.2 to 2.7, which mitigiated the serious corrosion concerns. It also protected more cellulose and hemicelluloses from extensive hydrolysis and further degradation to produce inhibitors as well as lignin condensations (*70*). The degree of dissolution of hemicelluloses, degradation of cellulose, sulfonation, and the condensation of lignin are increased as the reaction time and temperature increase and the pH decreases in the sulfite process (*13*). The results from another SPORL and dilute acid pretreatment on pine species under the conditions of 180 ºC for 30 min are listed in Table VI (kg/tone wood).

As can be seen, the SPORL pretreatment was more efficient than the dilute acid treatment for recovering sugars from the solid substrate and hydrolysate. The lignin in the hydrolysate of the SPORL process could be a potential by-product. Furthermore, the SPORL pretreatment at a higher pH (4.2) caused a higher substrate yield (solid content), but a lower enzymatic hydrolysis yield than that at a pH of 1.9. Otherwise, the total yield for both of the SPORL pretreatments was similar at different pHs. The glucan, xylan, mannan were higher, but the lignin was lower, in the substrate of the SPORL at a higher pH (4.2) (*8*).

Similar to chemical sulfite pulping process, inhibitors are generated during the pretreatment under dilute acid or the SPORL system. The concentration of inhibitors produced in the dilute acid and the SPORL pretreatments for spruce species are listed in Table VII (*70*).

Evidently, all inhibitors were more in the hydrolysate from the dilute acid pretreatment, but the concentration of lignin was higher in the hydrolysate of the SPORL pretreatment. The SPORL process produces lower amounts of acetic acid, hydroxymethylfurfural (HMF), and furfural than the dilute acid treatment does, because of the higher pH (*7*). Due to the lower inhibitors presented in the hydrolysate, the ethanol production would be facilitated with the SPORL system (*70*).

The ethanol production is also influenced by the chemical pretreatment conditions in the SPORL system. The ethanol production from the substrate and hydrolysate of pine species under various SPORL pretreatment conditions at 180 ºC for 25 min and then fermenting via using *S. cerevisiae* (ATCC 200062) at 32 ºC for 72 h at 100 rpm are listed in Table VIII (*9*).

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Table VII. Concentration of inhibitors induced during the chemical pretreatment of dilute acid or SPORL system for spruce species. (Reproduced with permission from reference (*70*). Copyright 2010 Elsevier)

Hydrolysate	*Acid-soluble lignin, g/l*	*Formic acid, g/l*	*Acetic acid, g/l*	*Furfural, g/l*	*HMF, g/l*	*Levulinic acid, g/l*
Dilute acid	4.8	7.4	5.3	2.9	4.7	11.4
SPORL	16.6	1.9	2.7	1.3	2.0	3.2

Table VIII. Ethanol production in the SPORL system of pine species under various conditions. (The data in parenthesis represents the ethanol yield production based on theoretical calculation). (Reproduced with permission from reference (*9*). Copyright 2010 Springer)

Acid charge, %	*Bisulfate charge, %*	*Initial pH*	*Substrate ethanol*[1]	*Substrate ethanol efficiency*[2]	*Hydrosate ethanol*[1]	*Hydrolysate ethanol efficiency*[2]	*Total ethanol yield*
2.21	4	1.8	136.7	73.7	52.8	65.1	189.5(49.2)
2.21	8	1.9	209.4	83.8	66.9	94.2	276.3(71.7)
1.4	8	2.3	193.2	76.4	36.3	70.4	229.5(59.6)
0	8	4.2	165.6	69.6	1.7	11.3	166.7(43.3)

[1] l/ton wood. [2] percentage of the theoretical yield.
3:l/ton of wood.

As can be seen, by increasing the bisulfite concentration from 4% to 8%, the ethanol production and efficiency were increased for both the substrate and hydrolysate, resulting in 21% increase in the total ethanol production. It is also noticeable that the ethanol production from the substrate or hydrolysate was reduced by reducing the acid charge (increasing pH) of the pretreatment.

References

1. Gullichsen, J.; Fogelholm, C. J. In *Papermaking Science and Technology, Chemical Pulping*, Book 6; Gullichsen, J., Paulapuro, H., Fapet Oy, Jyvaskyla, Finland, 1999.
2. Sixta, H. In *Hand Book of Pulp*, 1st ed.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2006; volume 1.
3. Janzon, R.; Puls, J.; Saake, B. *Holzforschung* **2006**, *60*, 347–354.
4. Helle, S. S.; Murry, A.; Lam, J.; Cameron, D. R.; Duff, S. J. B. *Bioresour. Technol.* **2004**, *92*, 163–171.
5. Bajwa, P. K.; Shireen, T.; D'Aoust, F.; Pinel, D.; Martin, V. J. J.; Trevors, J. T.; Lee, H. *Biotechnol. Bioeng.* **2009**, *104* (5), 892–897.

6. Taherzadeh, M. J.; Fox, M.; Hjorth, H.; Edebo, L. *Bioresour. Technol.* **2003**, *88*, 167–77.
7. Zhu, J. Y.; Pan, X. J. *Bioresour. Technol.* **2010**, *101*, 4992–5002.
8. Zhu, W.; Zhu, J. Y.; Gleisner, R.; Pan, X. J. *Bioresour. Technol.* **2010**, *101*, 2782–2792.
9. Zhu, J. Y.; Zhu, W.; O'Bryan, P.; Dien, B. S.; Tian, S.; Gleisner, R.; Pan, X. J. *Appl. Microbiol. Biotechnol.* **2010**, *86*, 1355–1365.
10. Magdzinski, L. *Pulp Pap. Can.* **2006**, *107* (6), 44–46.
11. Lawford, H. G.; Rousseau, J. D. *Appl. Biochem. Biotechnol.* **1993**, *39/40*, 667–685.
12. Helle, S. S.; Lin, T.; Duff, S. J. B. *Enzyme Microbiol .Technol.* **2008**, *42*, 259–264.
13. Bryce, J. R. G. Sulfite Pulping. In *Pulp and Paper Chemistry and Chemical Technology*, 3rd ed.; John Wiley & Sons: New York, 1980, pp 291−376.
14. Li, H.; Saeed, A.; Ni, Y.; van Heiningen, A. R. P. *J. Wood Chem. Technol.* **2010**, *30* (1), 48–60.
15. Area, M. C.; Felissia, F. E.; Nunez, C. E.; Venica, A.; Valade, J. L. *Cellulose Chem. Technol.* **2000**, *34*, 173–182.
16. Area, M. C.; Felissia, F. E.; Nunez, C. E.; Venica, A.; Valade, J. L. *Cellulose Chem. Technol.* **2000**, *34*, 525–535.
17. Livingstone, L. A. Pulping Conference, Proceedings of the Technical Association of the Pulp and Paper Industry, 2001, pp 273−287.
18. Shen, K. C.; Calve, L. *Adhesive Age* **1980**, 25–29.
19. Collins, J. W.; Boggs, L. A.; Webb, A. A.; Wiley, A. A. *Tappi* **1973**, *56* (6), 121–124.
20. Ringena, O.; Saake, B.; Lehnen, R. *Holzforschung* **2005**, *59*, 604–611.
21. Bansal, I. K.; Wiley, A. J. *Tappi* **1975**, *58* (1), 125–130.
22. Fredheim, G. E.; Braaten, S. M.; Christensen, B. E. *J. Wood Chem. Technol.* **2003**, *23* (2), 197–215.
23. Ekeberg, D.; Gretland, K. S.; Gustafsson, J.; Braten, S. M.; Fredheim, G. E. *Anal. Chim. Acta* **2006**, *565*, 121–128.
24. Chakrabarty, K.; Krishna, K. V.; Prabirkumar, S.; Ghoshal, A. K. *J. Membrane Sci.* **2009**, *330*, 135–144.
25. Bansal, I. K.; Wiley, A. J. *Current Res.* **1974**, *8* (13), 1085–1090.
26. De Wilde, F. G. N. *Desalination* **1987**, *67*, 495–505.
27. Jonsson, A. S.; Wallberg, O. *Desalination* **2009**, *237*, 254–267.
28. Ringena, O.; Saake, B.; Lehnen, R. *Holzforschung* **2005**, *59*, 405–412.
29. Restolho, J. A.; Prates, A.; de Pinho, M. N.; Afonso, M. D. *Biomass Bioeng.* **2009**, *33*, 1558–1566.
30. Gaddis, J. L.; Fong, D. S.; Tay, C. H.; Urbantas, R. G.; Lindgren, D. *Tappi J.* **1991**, *74* (9), 121–124.
31. Bhattacharya, P. K.; Todi, R. K.; Tiwari, M.; Bhattacharjee, C.; Bhattacharjee, S.; Datta, S. *Desalination* **2005**, *174*, 287–297.
32. Chakrabarty, K.; Saha, P.; Ghoshal, A. K. *J. Membrane Sci.* **2010**, *346*, 37–44.
33. Chakrabarty, K.; Saha, P.; Ghoshal, A. K. *J. Membrane Sci.* **2010**, *360*, 34–39.

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Publication Date (Web): July 11, 2011 | doi: 10.1021/bk-2011-1067.ch016

34. Lin, S. Y. Commercial Spent Pulping Liquor. In *Methods in Lignin Chemistry*; Lin, S. Y., Dence, C. W. Springer: Berlin, 1992, pp 75−80.
35. Eisenbraun, E. W. *Tappi* **1963**, *2*, 104–107.
36. Haars, A.; Lohner, S.; Huttermann, A. *Holzforschung* **1981**, *3*, 59–65.
37. Konturri, A. K.; Sundholm, G. *Acta. Chem. Scand.* **1986**, *40*, 121–125.
38. Forss, K.; Kokkonen, R.; Sirelius, H.; Sagfors, P. E. *Pulp Pap Can.* **1979**, *80* (12), 107–112.
39. Pearl, I. R.; Olcay, A. *Tappi* **1971**, *54* (10), 1656–1658.
40. Bryan, C.; Can, C. U. S. Patent 528837, 1956.
41. Forss, K. G.; Talka, E. T.; Fremer, K. E. *Ind. Eng. Chem. Res.* **1986**, *25*, 103–108.
42. Kelly, C.; Jones, O.; Barnhart, C.; Lajoie, C. *Appl. Biochem. Biotechnol.* **2008**, *148*, 97–108.
43. Rodrigues, R. C. B.; Lu, C.; Lin, B.; Jeffries, T. W. *Appl. Biochem. Biotechnol.* **2008**, *148*, 199–209.
44. Mikkola, J. P.; Sjoholm, R.; Salmi, T.; Maki-Arvela, P. *Catal. Today* **1999**, *28*, 73–81.
45. Mikkola, J. P.; Vainio, H.; Salmi, T.; Sjoholm, R.; Ollonqvist, T.; Vayrynen, J. *Appl. Catal., A* **2000**, *196*, 143–155.
46. Mikkola, J. P.; Salmi, T. *Catal. Today* **2001**, *64*, 271–277.
47. Granstrom, T. B.; Izumori, K.; Leisola, M. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 273–276.
48. Vandeska, E. A. S.; Kuzmanova, S.; Jeffries, T. W. *World J. Microb. Biotechnol.* **1995**, *11*, 213–218.
49. Rodrigues, R. C.; Sene, L.; Matos, G. S.; Roberto, I. C.; Pessoa, A., Jr.; Felipe, M. G. *Curr. Microbiol.* **2006**, *11*, 53–59.
50. Kim, T. B.; Oh, D. K. *Biotechnol. Lett.* **2003**, *25*, 2085–2088.
51. Oh, D. K.; Kim, S. Y.; Kim, J. H. *Biotechnol. Bioeng.* **1998**, *58*, 440–444.
52. Parajo, J. C.; Dominguez, H.; Dominguez, J. M. *Bioresour. Technol.* **1998**, *66*, 25–40.
53. Walthers, T.; Hensirisak, P.; Agblevor, A. F. *Appl. Biochem. Biotechnol.* **2001**, *91-93*, 423–435.
54. Silva, C. J. S. M.; Roberto, I. C. *Proc. Biochem.* **2001**, *36*, 1119–1124.
55. Rao, R. S.; Jyothi, C. P.; Prakasham, R. S.; Sarma, P. N.; Rao, L. V. *Bioresour. Technol.* **2006**, *97*, 1974–1978.
56. Sene, L.; Felipe, M. G. A.; Vitolo, M.; Silva, S. S.; Mancilla, I. M. *J. Basic Microbiol.* **1998**, *38*, 61–69.
57. Martin, C.; Marcet, M.; Almazan, O.; Jonsson, L. F. *Bioresour. Technol.* **2007**, *98*, 1767–1773.
58. Chipeta, Z. A.; Du Preez, J. C.; Szakacs, G.; Christopher, L. *Appl. Microbiol. Biotechnol.* **2005**, *69* (1), 71–78.
59. Linden, T.; Peetre, J.; Hahn-Hagerdal, B. *Appl. Environ. Microbiol.* **1992**, *58* (5), 1661–1669.
60. Helle, S.; Cameron, D.; Lam, J.; White, B.; Duff, S. *Enzyme Microb. Technol.* **2003**, *33*, 786–792.
61. Keating, J. D.; Robinson, J.; Cotta, M. A.; Saddler, J. N.; Mansfield, S. D. *J. Ind. Microbiol. Biotechnol.* **2004**, *31*, 235–244.

62. Nigam, J. N. *J. Ind. Microbiol. Biotechnol.* **2001**, *26*, 145–150.
63. Xavier, A. M. R. B.; Correia, M. F.; Pereira, S. R.; Evtuguin, D. V. *Bioresour. Technol.* **2010**, *101*, 2755–2761.
64. Schneider, H. *Enzyme Microbiol. Technol.* **1996**, *19*, 94–98.
65. Area, M. C.; Felissia, F. E.; Nunez, C. E.; Venica, A.; Valade, J. L. *Cellulose Chem. Technol.* **2000**, *34*, 173–182.
66. Helle, S. S.; Petretta, R. A.; Duff, S. J. B. *Enzyme Microb. Technol.* **2007**, *41* (1-2), 44–50.
67. Zhu, J. Y.; Pan, X. J.; Wang, G. S.; Gleisner, R. *Bioresour. Technol.* **2009**, *100*, 2411–2418.
68. Wang, G. S.; Pan, X. J.; Zhu, J. Y.; Gleisner, R.; Rockwood, D. *Biotechnol. Prog.* **2009**, *25* (4), 1086–1093.
69. Tian, S.; Luo, X. L.; Yang, X. S.; Zhu, J. Y. *Bioresour. Technol.* **2010**, *101*, 8678–8685.
70. Shuai, L.; Yang, Q.; Zhu, J. Y.; Lu, F. C.; Weimer, P. J.; Ralph, J.; Pan, X. J. *Bioresour. Technol.* **2010**, *101*, 3106–3114.

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Chapter 17

Integrated Forest Biorefineries – Near-Neutral Process

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Integrated forest biorefineries (IFBRs) have great potential for economic production of biofuels, chemicals and biomaterials in addition to paper, board and wood products. The favorable techno-economics result from capital and operating cost savings due to availability of wood and biomass collection and processing facilities, water treatment and boiler systems, permits and labor.

One promising version of an IFBR is the so called "near-neutral green liquor process" where hemicelluloses are pre-extracted using green liquor prior to conventional alkaline pulping of hardwood chips. The extract is converted into acetic acid and biofuels such as ethanol or butanol. Its attraction is the integrated production of renewable biofuels and chemicals, while the pulp yield and properties remain similar to conventional kraft pulp. Also the lime kiln is off-loaded because less white liquor is used for pulping. However, the process has higher energy demand because the organics in the extract are now not used for energy generation.

In this paper the effect of feedstock type and pre-extraction operating conditions on the extract composition and pulp yield and quality are reviewed. The techno-economics are presented.

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

Introduction

Forest biorefineries are now recognized as the best path forward to provide bioenergy, address the economic viability of the forest products industry in temperate climates, and reduce greenhouse gas emissions (*1*). This study concludes that several bio-energy and bio-product technologies are stronger economically when integrated within the wood products and pulp industry rather than on a stand-alone basis. It is also found that for these integrated technologies the employment multiplier ratios are much higher than most non-integrated bio-energy options (*1*).

The integration of new bio-energy pathways with existing pulp and (engineered) wood mills reduces the capital cost barrier for these new technologies because existing combustion facilities, water and effluent treatment and some chemical operations of the mills may be used. If these bioproducts are commodity products such as ethanol, butanol, diesel fuel and acetic acid, then presently they should be derived from hemicelluloses and lignin and not from cellulose. The reason is that hardwood pulp, consisting domininantly of cellulose, fetches a market price of $500-$1000/oven dry metric ton (ODMT)), while a price of ethanol of US$1.50/gallon provides a value of only about $200/ODMT when the entire pulp is converted to ethanol at a mass yield of about 40%. Thus it is clear that presently the traditional forest products should remain at the core of a forest biorefinery, with the new products being produced from the non-cellulose part of the wood chips or from additional cheaper biomass. Only when the new products have a significantly higher price than their fuel value, and have a market size similar to or larger than that of forest products, a paradigm shift away from traditional products will occur. (*2*).

Forest Biorefinery Pathways

Shown in Figure 1 are several different forest biorefinery pathways which are integrated with an existing forest products mill. On the left of the diagram the different feedstocks are listed including (pulp/lumber) wood extract and pulping liquor created as intermediary streams in the traditional pulp/wood products mill. The other four (4) feedstocks are forest biomass, MSW, agro-lignocellulosics (straw/corn stover, etc) and sludges. The diagram shows five pathways to fuels and chemicals listed on the right.

In Figure 1 there are two pathways which volatilize the entire feedstock (thermochemical conversion) either to syngas ($CO + H_2$) by partial oxidation or to pyrolysis-oil by thermal treatment in the absence of oxygen. The three other pathways generate intermediates by liquid phase processing. The highest severity treatment (in terms of temperature and acidity), leads to dehydration of the C6 and C5 sugars to levulinic acid and furfural respectively. A lower severity acid treatment leads to hydrolysis of the carbohydrate polymers to (C6 and C5) mono sugars and acetic acid. Finally the last pathway called consolidated fermentation is slow anaerobic fermentation by a natural mixture of microbes which produce organic acids and finally methane at a near neutral pH maintained by adding a base such as $Ca(OH)_2$. Since none of the chemical mixtures formed by these

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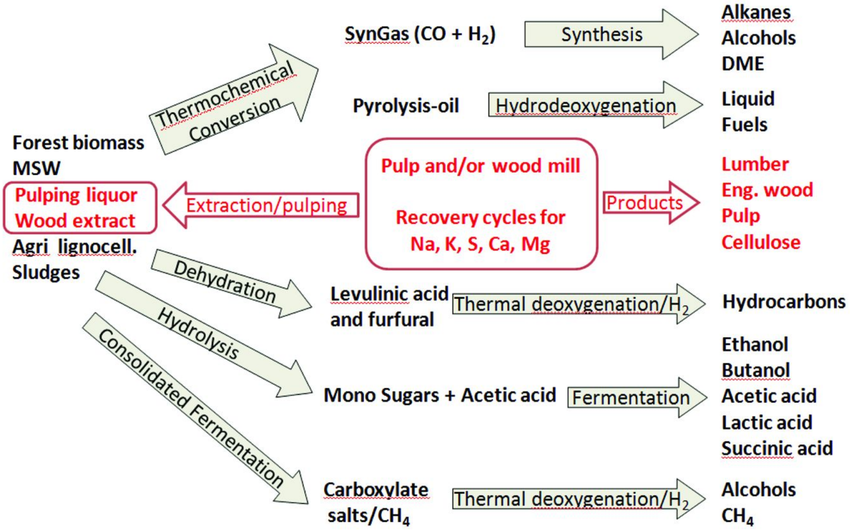
five pathways are marketable products, they need to be upgraded to chemicals or liquid fuels by decreasing the oxygen content. Another important difference between the pathways is that the bottom three do not utilize lignin in final products, while the top two do. Since lignin represents about 37% of the energy in softwoods and about 29% in hardwoods (*3*), this may be a disadvantage if the energy requirement of the conversion processes is less than that of the energy content of lignin (if lignin is used for energy generation).



SynGas (CO + H_2)
Synthesis
Alkanes
Alcohols
DME
Thermochemical Conversion
Pyrolysis-oil
Hydrodeoxygenation
Liquid Fuels
Forest biomass
MSW
Pulping liquor
Wood extract
Agri lignocell.
Sludges
Extraction/pulping
Pulp and/or wood mill
Recovery cycles for Na, K, S, Ca, Mg
Products
Lumber
Eng. wood
Pulp
Cellulose
Dehydration
Levulinic acid and furfural
Thermal deoxygenation/H_2
Hydrocarbons
Hydrolysis
Mono Sugars + Acetic acid
Fermentation
Ethanol
Butanol
Acetic acid
Lactic acid
Succinic acid
Consolidated Fermentation
Carboxylate salts/CH_4
Thermal deoxygenation/H_2
Alcohols
CH_4

Figure 1. Forest biorefinery pathways.

Wood Extraction and Kraft Pulping

The wood extract in Figure 1 is obtained by pretreatment of wood chips or wood strands prior to pulping or production of oriented strand board (*4*) respectively. The aqueous solution used for extraction may be pure water, acidic or alkaline. Steam is used in the prehydrolysis-kraft process for production of dissolving pulp (*5*). However, this is not part of the Biorefinery concept in Figure 1 since the wood extract is combusted together with the spent kraft pulping liquor. Pure water is preferred because it is cheap and environmentally friendly and results in simpler downstream processing compared to acid and alkaline pre-hydrolysis. Water pre-extraction is also called autohydrolysis because dissolution of wood components is catalysed by acetic acid obtained by hydrolysis of acetyl groups in the hemicelluloses which lowers the pH of the extract to 3~4 (*6*, *7*). Autohydrolysis in combination with kraft pulping to produce co-products such as ethanol besides pulp has been reported by several research groups (*8*–*11*). However, as a result of the acidic conditions, high temperature pre-extraction with water leads to a significant loss in pulp yield and paper strength in the subsequent pulping step (*11*, *12*). Pre-extraction of Southern or Loblolly Pine chips with dilute acid followed by kraft pulping was studied by Frederick (*13*) and

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Yoon (*12*). Both report large losses in pulp yield. Pre-extraction of poplar wood chips at strong alkaline conditions (1-2 Molar NaOH at L/W of 4 kg/L) and low temperatures (50 – 90°C) followed by kraft pulping does not lead to a loss in pulp yield and properties (*14*, *15*). At these conditions about 40-50 kg (per metric tonne of oven dry original wood) of hemicellulose oligomeric sugars could be extracted without detrimental effect on overall pulp yield when the extracted wood was subjected to modified kraft pulping. In the so-called "near neutral" hemicellulose extraction process, the subject of this review, hardwood chips are extracted with alkaline solutions at low alkali charges and high temperatures (160-170 °C) so that the pH of the final extract is near neutral. When the "near-neutral" extraction process is followed by kraft pulping again no significant loss in pulp yield and properties was observed (*16–18*). The lignocellulosic biorefinery has been simulated in a number of studies producing pulp and ethanol (*19*) butanol (*20*) or lactic acid (*21*). Finally, an interesting process, not covered in Figure 1, has recently been described (*22*, *23*) which produces only ethanol by cooking wood chips in green liquor (principally Na_2CO_3 and Na_2S) followed by mechanical refining and then enzymatic hydrolysis and fermentation of the high lignin and hemicellulose containing fibers. This process is economical because the entire infrastructure of a non-profitable kraft pulp mill is used at negligible cost, thereby significantly reducing capital cost.

Near Neutral Hemicellulose Extraction Concept

In 2004 initial pre-extraction/kraft studies were performed on mixed Southern Hardwood chips (35% Gum, 35% Southern Red Oak, 15% Red Maple, 12% Poplar and Sycamore and 3% Southern Magnolia) at the University of Maine. The chemical composition of the southern hardwoods mixture (SHM) is given in Table 1.

These initial experiments established that both pure water and strong alkaline pre-extraction (10% NaOH as g Na_2O/100 g wood) at a liquor-to-wood (L/W) ratio of 4.5 L/kg before pulping led to a pulp yield loss (on wood) of 5% or more relative to the control kraft cook (*24*) Water extraction at 150 °C for 90 minutes dissolved 12 % of the wood at a final extract pH of 3.7 while pre-extraction at a charge of 10% NaOH at 125 °C for 45 minutes dissolved 17% of the wood in a final extract with pH 13.3. The reduction in total pulp yield for pure water extraction was mostly due to additional xylan loss, while the kraft pulp yield loss after 10% NaOH pre-extraction was caused by both xylan and cellulose loss. However, the pulp yield loss compared to the control was only 1% when the NaOH charge during pre-extraction at 150 °C for 90 minutes was reduced to 3 %. About 8% of the wood was dissolved during pre-extraction in the extract with a final pH of 6.2. Thus it was found that the final extract pH must be near neutral at high temperature pre-extraction to avoid significant total pulp yield loss. This treatment was then called "near-neutral" pre-extraction (*25*).

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

Table 1. Chemical composition of southern hardwood mixture (% on extractives-free wood)

Chemical component	Amount (% by wt)	Chemical component	Amount (% by wt.)
Arabinan	0.52±0.01	Klason Lig.	25.8±0.2
Galactan	1.00±0.02	Acid Sol. Lig.	3.20±0.05
Glucan	43.7±0.6	Ash	0.34±0.07
Xylan	15.6±0.3	AcG	2.76±0.08
Mannan	2.2±0.1	UAG	4.8±0.10

AcG : Acetyl groups UAG : Uronic acid groups

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Development of Near-Neutral Green Liquor (NNGL) Process

Practical implementation of this “near-neutral” pre-extraction treatment favors the use of mill-available alkali sources such as green liquor or Na_2CO_3. Therefore a new set of pre-extraction experiments was performed on the SHM (Table 1) with green liquor (GL) + anthraquinone (AQ). The alkali charge during pre-extraction was 3% on wood (as total titratable alkali or TTA expressed in g Na_2O), while AQ was added at a charge of 0.05% on OD wood to further improve the final pulp yield. No AQ was added during cooking, including the control kraft cook. The sulfidity of the green liquor was 30% on TTA basis (*24*, *25*). Pre-extraction for 90 minutes at 140 °C (H-factor of 106 hr) , 60 minutes at 160 °C (H-factor of 403 hr), and 110 minutes at 160 °C (H-factor of 792 hr) at L/W=4.5 L/kg led to dissolution of 4.5, 8.3 and 9.6 % of the wood respectively. The residual pH of the 3% GL (+AQ) extraction liquor is plotted as a function of extraction H-factor in Figure 2. It shows a rapid drop from the initial alkaline green liquor pH to a near-neutral value of around 6.5 at an H-factor of about 100 hours and then steadily drops below pH 5.5 at H-factors larger than about 800 hours. The pH drop is mostly caused by continued formation of acetic acid by hydrolysis of the acetyl groups in hemicellulose.

The composition of the near-neutral extracts using 3% GL (+AQ) at the three different H-factor conditions (106, 403 and 792 hr) is given in Table 2. In all cases, acetate is the largest organic component in the extract followed by lignin. Xylan increases strongly with increasing H-factor and reaches 1.45 % (on oven dry wood) at H=792 hrs. It should be noted that the identified organics in the extract represent about 60% of the wood weight loss, i.e. 40% of the dissolved wood is not identified. The unidentified organics are degraded hemicelluloses formed during high temperature treatment at the initially slightly alkaline conditions. Additionally, the amount of dissolved xylan is underestimated because xylan is quantified as xylose by HPAEC analysis after acid hydrolysis, and this treatment cleaves only 40% of the linkages between xylan and glucuronic acid side-groups (*26*, *27*).

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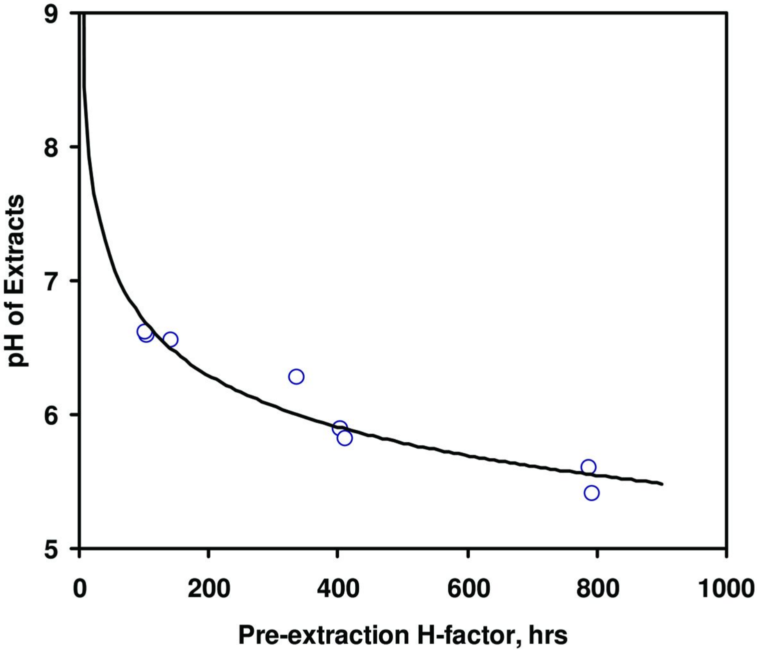

Figure 2. pH of 3% GL (+AQ) extracts versus pre-extraction H-factor.

The kraft delignification rate of the control and 3% GL (+AQ) pre-extracted SHM chips is seen in Figure 3 as kappa number versus kraft pulping H-factor. All pre-extraction kraft cooks showed higher delignification rates than the control cook. The increased delignification rate may be due to improved accessibility of the cell wall as well as cleavage of lignin-carbohydrate covalent bonds during pre-extraction (*28*). It should also be noted that the effective alkali (EA) charge during kraft cooking of the pre-extracted chips is reduced to 12% (as Na_2O on od wood) compared to 15% for the control.

Despite the 3% lower charge in EA, the residual EA concentration in the spent liquor was higher for the pre-extraction kraft cooks in the bleachable grade kappa range than that of the control as can be seen in Figure 4. Thus the green liquor active alkali requirements for pre-extraction are balanced by white liquor effective alkali consumption savings during kraft pulping.

The effect of pre-extraction on total pulp yield based on original wood weight is shown in Figure 5. Even though the data are scattered, it can be seen that the total pulp yield for the kraft cooks with near-neutral pre-extraction is equal to that of the control kraft pulps within ±1% in the bleachable grade kappa number range.

The papermaking properties of the near-neutral pre-extracted kraft pulps and the control kraft pulps were evaluated in terms of their refining response, tensile strength and tear resistance. Handsheets were prepared for kraft pulps with 3% GL (+AQ) pre-extraction at 400 H-factor and of conventional kraft pulps without pre extraction

Table 2. Composition of extraction with 3% GL (+AQ) of southern mixed hardwood chips for 90 minutes at 140 °C and 60 and 90 minutes at 160 °C

Composition of Extract	*% on ODW*		
	90min-140 °C (H-factor 106)	*60min-160 °C (H-factor 403)*	*110min-160 °C (H-factor 792)*
Wood Weight Loss	4.45	8.30	9.62
Xylan	0.26	0.70	1.40
Glucan	0.11	0.19	0.35
Mannan	0.05	0.05	0.08
Arabinan	0.03	0.06	0.10
Galactan	0.08	0.12	0.20
4-O-MGA	0.11	0.18	0.37
Acetate	1.45	2.04	2.08
Lignin	0.83	1.51	1.60
Total sugars	2.09	3.34	4.58
Dissolved organics	2.92	4.85	6.18

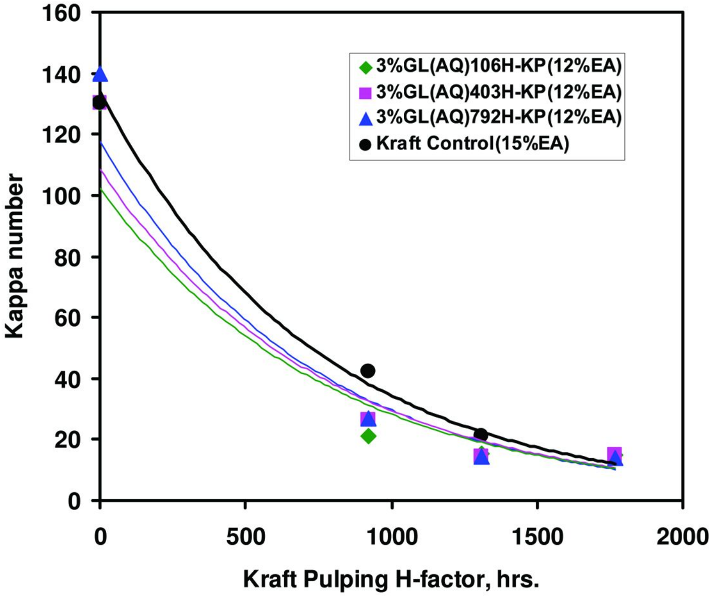

Figure 3. Kappa number versus kraft pulping H-factor.

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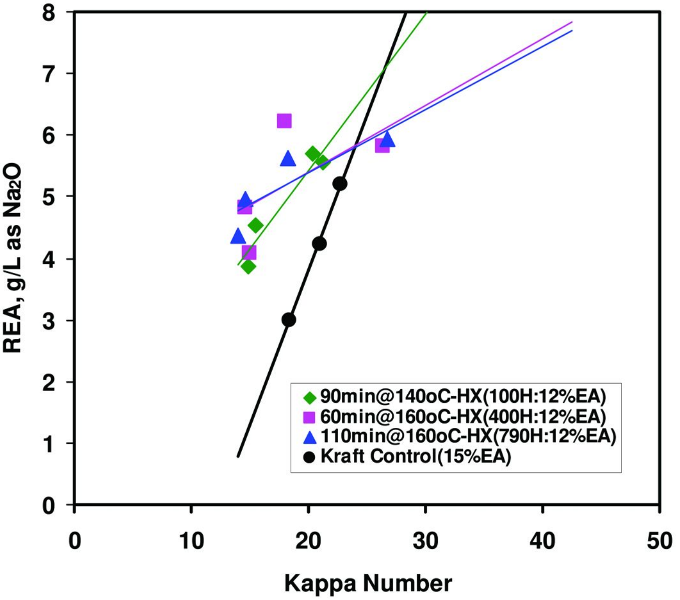

Figure 4. Residual effective alkali (REA) versus kappa number.

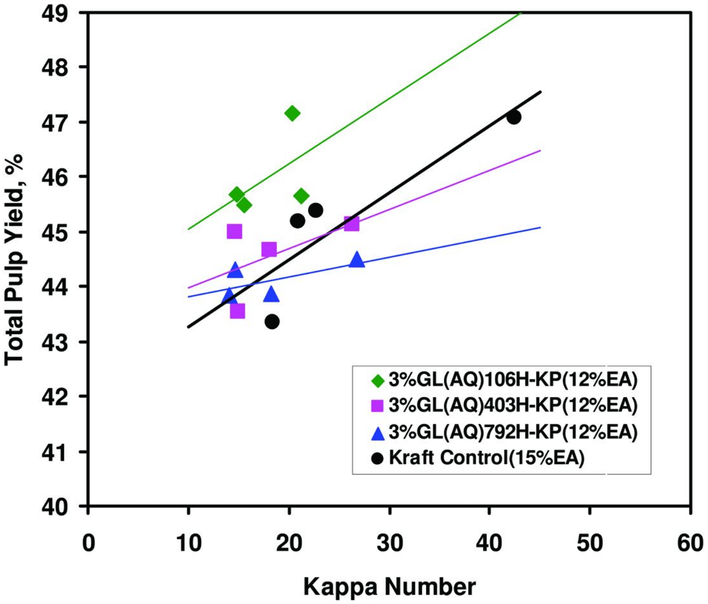

Figure 5. Total pulp yield versus kappa number for kraft cooks of 3% GL (+AQ) pre-extracted wood chips and of control kraft cooks.

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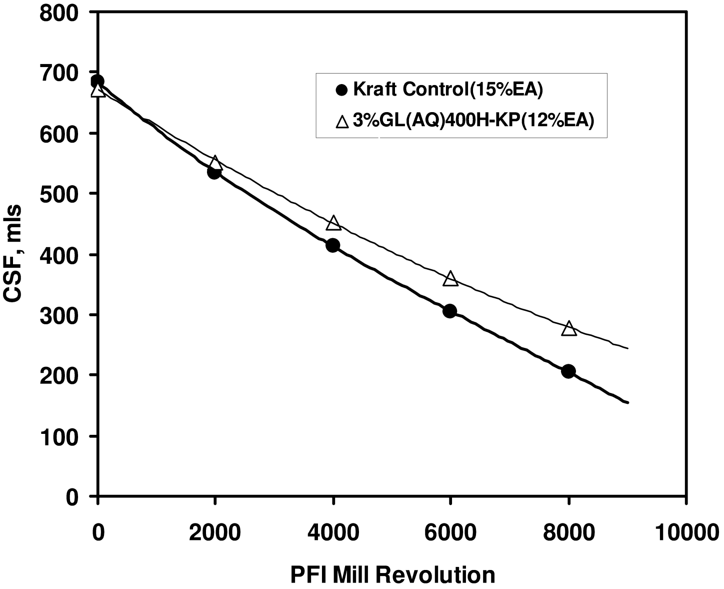

Figure 6. Pulp freeness (CSF) versus number of PFI mill revolutions for 3% GL (+AQ) 400H pre-extracted kraft pulps and control kraft pulps.

The PFI mill refining responses of the pulps are shown in Figure 6. It shows that the pre-extracted kraft pulps need slightly more beating to attain the same level of freeness.

In terms of paper strength, the tear resistance versus tensile strength of the handsheets prepared from the PFI mill refined pulps are displayed in Figure 7. It shows that a 5-10% higher tear resistance was observed for the "near-neutral" pre-extracted pulps compared to the control pulps. From these results it may be concluded that the "near-neutral" pre-extracted pulps are slightly more difficult to beat but have a significantly increased tear resistance relative to concentional kraft pulps made from the same hardwood chips. Exactly the same conclusion was reached in another recent study of the near-neutral pre-extraction kraft process performed on similar southern hardwood chips (*29*).

Effect of GL Charge on Extract Composition

In the previous section it was shown that a reasonable amount of wood sugars (3.3-4.6% on wood, Table 2) were present in the 3% GL extract after contacting hardwood chips at 160 °C and H-factor of 400-800 hr, and that the total pulp yield and properties were similar to that of the control kraft pulp. The NNGL process was then conducted on northeastern hardwood chips (50% maple, 25% beech, 15% birch and 10% aspen) to study the effect of green liquor (GL) charge on the extract composition. Green liquor charges of 0, 2, 4 and 6% on wood (TTA as Na_2O) at 160 °C and H-factor of about 800 hrs were used (*30*). 0.05% AQ (on wood) was also added during extraction to preserve pulp yield. The composition of the northeastern hardwood mixture is shown in Table 3.

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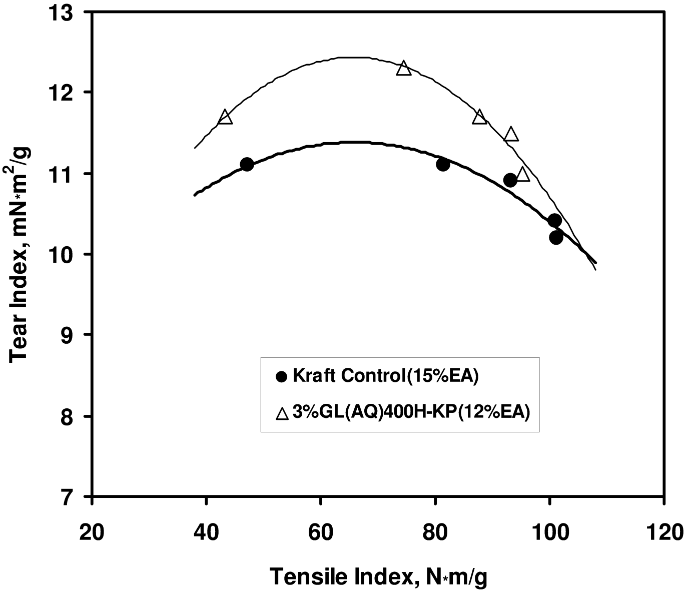

Figure 7. Tear index versus tensile index for 3% GL (+AQ) 400H pre-extraction kraft pulps and control kraft pulps.

Table 3. Composition of northeastern hardwood mixture (% on wood)

Chemical component	Amount (% by wt)	Chemical component	Amount (% by wt.)
Arabinan	0.56±0.01	Lignin	26.5±0.2
Galactan	1.22±0.02	Extractives	0.61±0.05
Glucan	42.7±0.6	Ash	0.51±0.05
Xylan	22.8±0.4	AcG	3.65±0.08
Mannan	2.23±0.05	UAG	2.8±0.1

AcG : Acetyl groups UAG : Uronic acid groups

The differences between the composition of the northeastern hardwood mixture (Table 3) and that of the southern hardwood mixture (Table 1) are mostly that the former has more acetyl groups (3.65 vs 2.76 %) and less uronic acids (2.8 vs 4.8%). The higher acetyl group content for northeastern hardwoods is advantageous when acetic acid is one of the co-products.

The extractions were performed for 110 minutes at 160 °C and L/W ratio of 4L/kg in a recirculating digester. With a 50 minute ramp to temperature this corresponds to an H-factor of 780 – 790. Then the digester was cooled below 100 °C and the liquor drained.

The digester pressure increases significantly above the saturated steam pressure during extraction due to CO_2 released from GL carbonate decomposition as may be seen in Figure 8. The hydroxide ions present in GL catalyze hydrolysis of the acetyl groups, and the released acetic acid then reacts with carbonate to form bicarbonate and acetate. When all carbonate (pKa = 10.3) is consumed, the formed bicarbonate (pKa = 6.35) decomposes to release CO_2 at near neutral pH by continued reaction with more released acetic acid. Thus at a pH below 6 most of carbonate in GL is released as CO_2. The pressure increase above saturated steam pressure at 4% GL charge (final extract pH of 5.26) was found to be about 80 psi, which is twice that when extracting with pure water. The latter pressure increase is also caused by CO_2 but now originating from decomposition of uronic acids in hardwood (*31*). The final pressure at 2% GL is similar to that obtained with pure water. Interestingly the pressure increase at 6% GL is similar to 2% GL because at the alkaline final pH not all bicarbonate is decomposed. There may also be a concern of release of H_2S from NaHS (pKa of 7.05) when the pH reaches near-neutral. However, the final extract liquor does not smell of H_2S, which suggests that the hydrosulfide anions reacts with lignin.

The compositions of the extracts are shown in Table 4. The composition is obtained from analysis of the liquid (shown under **Liq.**) and calculated from the wood analysis before and after extraction (under **Solid** which is abbreviated as **Sld.**). The final pH of the extract (bottom Table 3) varies between approximately 2.5 for extraction with pure water to 7.1 when using 6% GL. Only the latter shows a Total Inorganic Carbon (TIC) of 2.5g/L, explaining the earlier noted lower pressure increase in the digester at 6% GL.

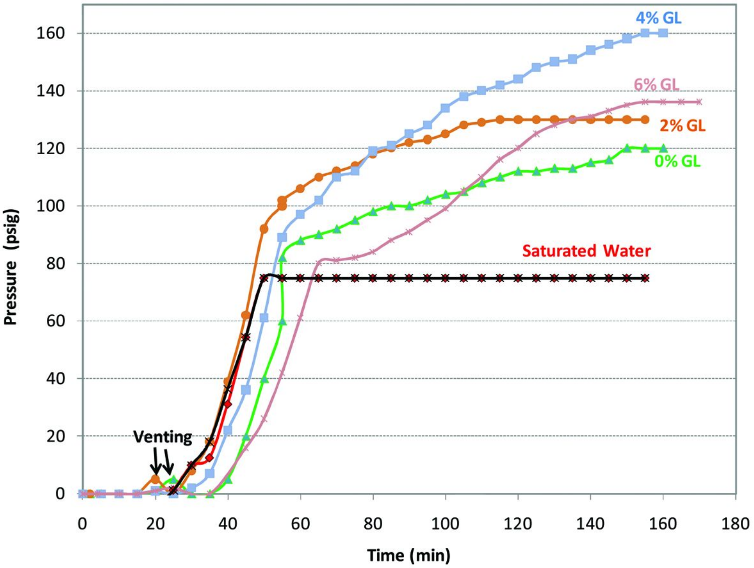

Figure 8. Total pressures during green liquor extraction (32).

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Based on the **Liq.** analysis, Table 3 shows that the acetic acid concentration increases from 3.7 to 11.8 g/L at GL charge from 0 to 6 % resp., in agreement with an increasing hydrolysis rate of acetyl esters with increasing alkalinity above pH of about 4 (*33*). It should be noted that for water extraction the acetic acid concentration more than doubles to 8.6 g/L if all acetyl groups still attached to dissolved xylan-oligomers are hydrolysed. For 2% GL extraction the acetic acid concentration increases from 8.5 to 9.8 g/L due to further hydrolysis, while the increases are insignificant for the two highest GL charges.

Table 4. Effect of green liquor charge on extract composition

Component (g/L)	*0% GL + 0.55% AQ*		*2% GL + 0.55% AQ*		*4% GL + 0.55% AQ*		*6% GL + 0.55% AQ*	
	Liq.	*Sld.*	*Liq.*	*Sld.*	*Liq.*	*Sld.*	*Liq.*	*Sld.*
Acetic acid	3.7		8.5		11.2		11.8	
Formic acid	0.93		1.77		2.42		3.71	
Lactic acid	0.0		0.04		0.83		1.73	
Furfural	2.39		0.70		0.32		0.18	
Lignin	5.58		5.17		6.31		9.24	
Xylan	22.0	26.1	5.92	13.2	3.59	10.6	2.36	10.8
Galactan+Rhamnan	1.47	1.87	0.57	1.47	0.47	1.27	0.44	1.49
Glucan	0.93	0	0.28	5.72	0.22	7.15	0.20	9.53
Mannan	1.57	1.33	0.22	1.34	0.15	2.06	0.11	2.73
Arabinan	0.68	1.15	0.31	0.98	0.24	0.59	0.21	0.48
Total Sugars	**26.7**	**29.8**	**7.30**	**22.7**	**4.67**	**21.7**	**3.32**	**25.0**
MeGlucuronic acid	0.51	6.12	0.29	4.59	0.22	3.85	0.13	3.76
Galacturonic acid	0.64		0.72		0.56		n.a.	
Glucuronic acid	0.39		0.20		0.11		n.a.	
Sodium	0.00		3.22		6.41		9.45	
Potassium	0.21		0.24		0.25		0.28	
Calcium	0.13		0.13		0.12		0.15	
Sulfur	0.00		0.22		0.52		0.93	
TIC (as CO_3)	0.05		0.05		0.07		2.50	
Protein (6.3xTN)	0.46		0.55		0.52		0.77	
Total SUM	**41.7**		**29.1**		**34.5**		**44.2**	

Continued on next page.

Table 4. (Continued). Effect of green liquor charge on extract composition

Component (g/L)	*0% GL + 0.55% AQ*		*2% GL + 0.55% AQ*		*4% GL + 0.55% AQ*		*6% GL + 0.55% AQ*	
	Liq.	***Sld.***	***Liq.***	***Sld.***	***Liq.***	***Sld.***	***Liq.***	***Sld.***
Exp. solids (g/L)	**42.9**		**32.6**		**37.3**		**53.6**	
Mass Balance (%)	**97.2**		**89.1**		**92.6**		**82.4**	
Final Extract pH	**2.46**		**4.26**		**5.26**		**7.11**	

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Both formic and lactic acid also increase with increasing GL charge, which is expected since both are known to be formed during alkaline degradation of sugars (*34*). Lactic acid is not formed during water extraction, indicating that it is only formed through alkaline degradation, while formic acid also has a smaller acidic formation route. Furfural has a concentration of 2.4 g/L in the water extract which is an order of magnitude higher than in the GL extracts due to its much lower pH. The lignin concentration is close to 6 g/L from 0 to 4 % GL, but then increases to 9.2 g/L at GL charge of 6%. The increase in soluble lignin concentration at the highest GL is due to the delignifying effect of Na_2S. The lignin concentration does not decrease at the lowest GL charge and for water extraction because more lignin is dissolved as LCCs (*28*).

The sugar concentration of the extracts expressed as anhydrosugars (i.e. xylan, mannan, etc) based on the liquor analysis shows that xylan is the major dissolved sugar. It decreases from about 22 g/L for the water extraction to only 2.4 g/L at 6% GL. The total sugar concentration decreases from 26.7 g/L for water extraction to 7.3 g/L at 2% GL to 4.7 g/L at 4% GL and finally 3.3 g/L at 6% GL. The large reduction in sugar extraction with addition of GL can partly be explained by the much lower acidity of these extracts compared to water extraction, i.e. autohydrolysis. However, this is only part of the explanation. The calculated sugar concentrations based on the analysis of the wood chips (i.e. under **Sld.**) shows that the total sugar concentration is about 30 g/L for the water extract, and about 22-25 g/L for the GL extracts, i.e. showing a much smaller drop. This suggests that a major part of the extracted sugars during GL extraction degrade by peeling reactions after their removal from wood (*34*). Specifically for xylan the difference between the **Liq.** and **Sld.** analysis suggests that about 7 g/L of xylan is lost by alkaline degradation and goes to other products.

The **Sld.** analysis of the sum of all uronic acids (measured by the method of Scott (*35*)) shows that the removal of uronic acids from wood decreases by almost half going from water to 6% GL extraction. If all the uronic acids would be removed from the original wood a concentration of about 7 g/L is calculated. This means that the uronic acids are almost completely removed during water extraction, while slighty less than half are removed at 6% GL. Analysis of the different uronic acids in the liquor (i.e. **Liq**) was performed by methanolysis-GC (*36*). This is necessary because the method of Scott (*35*) only works on solid wood or pulp. It can be seen that the sum of 4-O-methylglucuronic acid, glucuronic acid

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and the pectin galacturonic acid are much smaller than that obtained from the solid (i.e. **Sld.**) analysis. The difference can be explained by decarboxylation of the uronic acids during extraction, and is also in agreement with the pressure increase above the saturated steam temperature during extraction. The concentrations of sodium increase linearly with GL charge, while potassium and calcium remain unchanged This is expected because the former is part of GL while the latter two are part of the original wood. The sulfur content increases more than linear with GL charge because the sulfur is mainly associated with the lignin and the lignin concentration increases significantly at 6% GL charge. Finally it can be seen that the protein concentration in the extract is about 0.5 g/L. The presence of nitrogen in the extract may reduce the nitrogen addition required for fermentation of the sugars in downstream processing of the liquor.

Since the objective of the “near-neutral” green liquor process is to achieve as much xylan and acetyl group removal while maintaining the final kraft pulp yield and properties, these detailed results point to using the smallest GL charge still sufficient to minimize pulp yield loss.

Effect of GL Charge on Total Pulp Yield

The total pulp yields (i.e. based on original wood weight) at a total combined (extraction + kraft pulping) H-factor of about 1350 hrs for the different pre-extraction conditions are listed in Table 5. The effective alkali charge of 25% sulfidity white liquor during kraft pulping was adjusted to obtain a kappa number of about 18. Also included in Table 5 are two control kraft cooks (i.e without pre-extraction). It can be seen that the EA charge during pulping may be significantly reduced compared to the control kraft cooks. This is expected because both the acetyl groups and hemicellulose sugars removed during pre-extraction consume alkali during the kraft control cook.

Table 5. Effect of GL charge on total pulp yield

Green Liquor (%)	***Effective Alkali (%) during Pulping***	***H-Factor (Hrs)***	***Total Pulp Yield (%)***	***Kappa No.***	***Calculated Yield @ 18 Kappa (%)****
0	12	1363	**38.0**	15.2	38.8
2	12	1362	**46.9**	18.9	46.6
4	12	1368	**48.6**	**17.0**	48.5
6	10	1365	**49.7**	**18.4**	49.7
Kraft Control 1	14	**1313**	**50.8**	18.4	50.7
Kraft Control 2	16	**1368**	**48.6**	15.7	49.3

* Calculated as (Total pulp yield) + 0.3 x (18 – Kappa).

Table 6. Effect of L/W ratio on Extract Composition (H-factor of 800 hr)

GL (%)	L/W (L/kg)	Arabinose (g/L)	Glucose (g/L)	XMG (g/L)	Total sugars (g/L)	Acetic Acid (g/L)	Final pH	Stickyness of lignin
0	4	0.78	1.14	28.1	30.0	9.1	3.34	strong
0.5	4	0.41	0.73	22.1	23.2	7.9	3.95	strong
2	2.5	0.52	0.57	14.2	15.3	14.1	4.52	medium
2	4	0.46	0.34	9.6	10.4	10.3	4.77	light
2	5.5	0.35	0.27	9.4	10.0	7.1	4.86	light
4	4	0.36	0.26	5.3	6.0	11.6	5.64	none
6	4	0.26	0.23	2.9	3.4	12.5	8.91	none

Because the pulp yield is a function of kappa number, all the pulp yields are corrected to 18 kappa in the last column using the experience that each kappa number corresponds to 0.3% total yield. This column shows that the pulp yield of the control kraft cook on average is 50 %, which the same (within experimental error of ±0.5%) as that obtained at 6% GL. At 4 and 2% GL the total yield is 1.5 to about 3% lower than the control respectively. With pure water extraction the final pulp yield is 12% lower than the control. Thus the use of green liquor minimizes the total pulp yield loss, while water pre-extraction leads to an unacceptable loss.

Effect of L/W Ratio on Extract Composition

In a recent study (*37*) it was reported that xylan removal from hardwoods with water was twice as high in a throughflow reactor with very high final L/W ratio (600 L/kg) than in a batch reactor (L/W of 3.7 L/kg). Therefore another set of extraction experiments was performed whereby the L/W ratio as well as the GL charge were varied. The conditions and results are listed in Table 6. In all cases the extraction H-factor was about 800 hrs. The table shows the concentration of sugars and acetic acid after acid hydrolysis of the extracts. The last two columns show the extract pH measured at room temperature, and a measure of whether sticky lignin remained on the wood basket and digester after extraction.

Regarding the latter, it is seen that only at pH ≥ 5.0 the digester remains free of sticky lignin. This is a major advantage, since it has been reported (*38*) that water pre-hydrolysis of hardwood chips leads to formation of lignin-based precipitates which adhere to reactor walls and piping, and are very difficult to remove. These precipitates form when the temperature of the extract is lowered (due to the release of pressure), and also when the extracted hemicellulose oligomers are further hydrolyzed over time. This is the principal reason why hemicelluloses are not recovered in a commercial prehydrolysis-kraft process. The concentration of the removed sugars are plotted versus extract pH in Figure 9. It clearly shows that the concentration of all sugars (XMG is the sum of xylose, mannose and galactose) decreases in a smooth fashion with increasing pH irrespective of GL charge or L/W ratio (relative deviations less than 10%). However when the total amount of sugars removed is plotted as percentage on

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wood (see Figure 10) then it is seen that the extracts at L/W ratio of 2.5 and 5.5 L/kg deviate significantly (relative deviations of about 30%) from the general trend. The higher L/W ratio of 5.5 L/kg leads to more sugar removal than the trend line at L/W of 4 L/kg, while the opposite is true for the L/W ratio of 2.5 L/kg. This behavior may be explained as follows: the pH determines the amount of hydrolysis of the hemicellulose (and thus its degree of polymerization or DP), and the DP in turn determines the solubility of the hemicellulose fragments. Thus if the solution for dissolution is larger (i.e. L/W is larger), then more sugars may be removed from wood, and vice versa.

It should be noted that the sugar concentrations measured before acid hydrolysis are very small. Thus almost all sugars in the extract (except arabinose and galactose) are oligomers.

The same plots as for the sugars are shown for acetic acid in Figures 11 and 12. Contrary to the sugars it is found that there is a significant amount of acetic acid in the extract before acid hydrolysis, called free acetic acid (as sum of acetate + acetic acid). The total acetic acid concentration measured after full acid hydrolysis of the extract is called total acetic acid.

The results in Figure 11 show that the concentration of total acetic acid has a minimum at pH 4 and reaches a plateau of 12±0.5 g/L at pH 6 or higher. Considering that the original wood contains 3.65 % as acetyl groups, the total acetic acid concentration upon complete hydrolysis would be 12.7 g/L. Thus at pH 6 or higher the hydrolysis of the acetyl groups in wood proceeds to completion at 160 °C and 800 H. The minimum in total acetic acid concentration of about 8 g/L at pH 4 may be explained that alkaline catalysed hydrolysis increases at pH values above the pKa of acetic acid, while acid catalysed hydrolysis increases below the acetic acid pKa (*33*). The free acetic acid concentration is essentially the same as the total acetic acid concentration at about pH 5 or higher. This means that all dissolved sugar oligomers are deacetylated. Only at pH less than 4 the free acetic acid concentration is less than the total acetic acid concentration. Thus the sugar oligomers in the water extract still are partially acetylated, and about half of the removed acetyl groups are attached to the oligomers and half is free. It can also be seen in Figure 11 that the acetic acid concentration at L/W ratio of 2.5 L/kg is about 55 % higher than that at a L/W ratio of 4.0 at the same pH., while it is about 25% lower at L/W of 5.5 L/kg. This is expected based on the volume of extract available for dissolution of acetic acid. A confirmation that the amount of acetic acid generated is not a function of L/W ratio is seen in Figure 12 where the amount of acetic acid in the extract is calculated based on the amount of original wood.

It can be seen that the effect of L/W is mostly eliminated, showing that the rate and amount of acetic acid production is governed only by the final pH. This behavior is different from dissolution of the sugar oligomers which is also affected by oligomer solubility.

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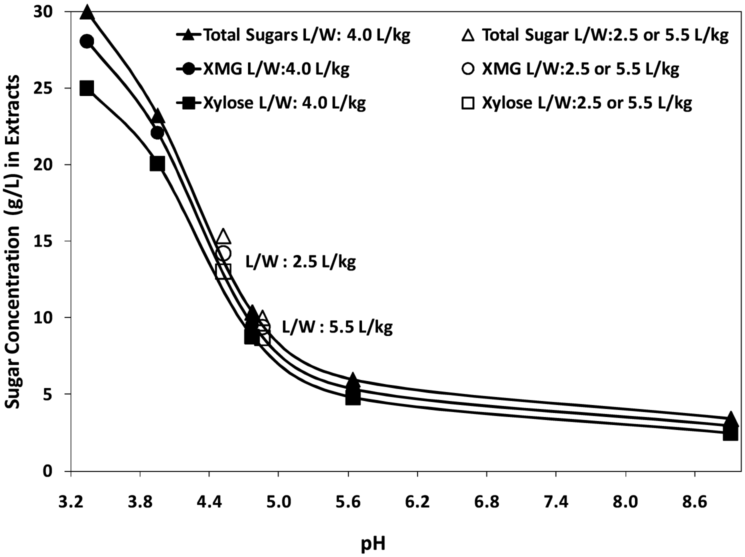

Figure 9. Sugar concentration versus pH at different GL charge and L/W ratios (see Table 6).

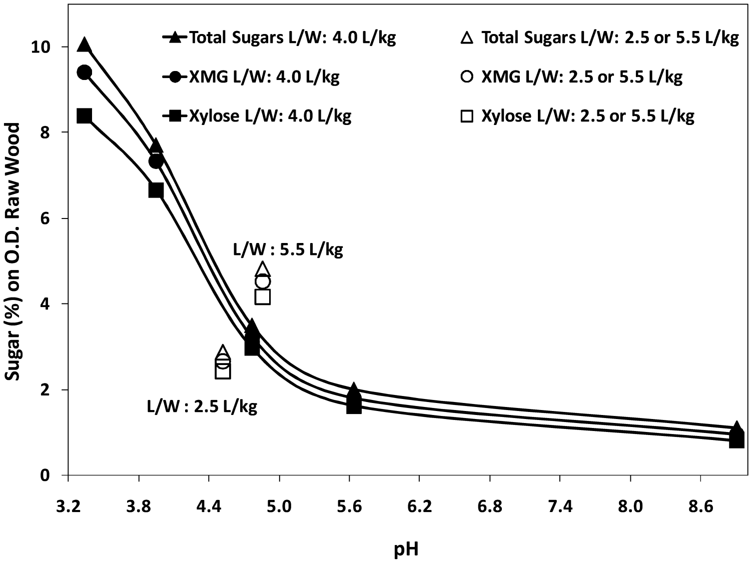

Figure 10. Sugar removal (% on wood) versus pH at different GL charge and L/W ratios (see Table 6).

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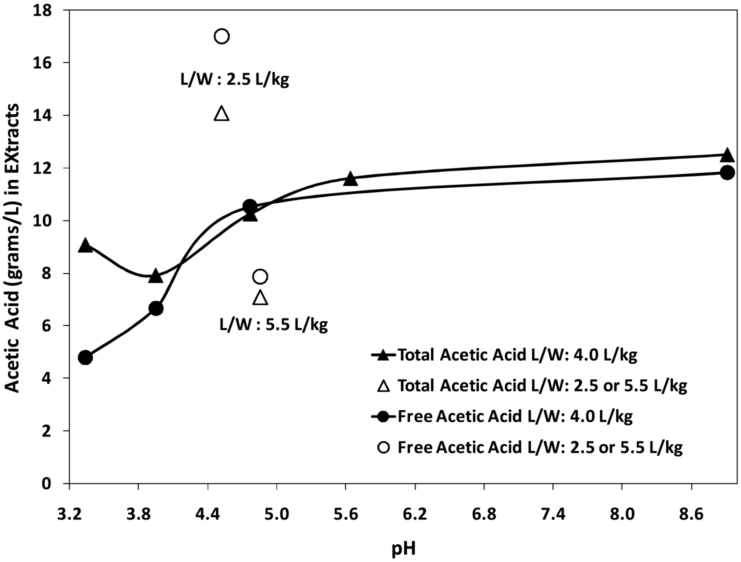

Figure 11. Acetic acid concentration versus pH at different GL charge and L/W ratios (see Table 6).

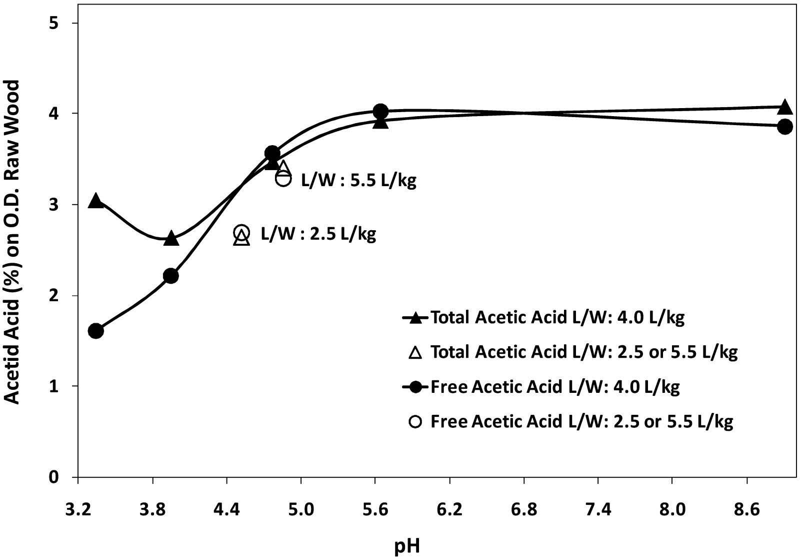

Figure 12. Acetic acid removal (% on wood) versus pH at different GL charge and L/W ratios (see Table 6).

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Technical Economic Analysis

Process Description

A detailed techno-economic analysis of the near neutral hemicellulose extraction process integrated in a kraft mill has been presented by Mao and co-workers (*19*). The process in Figure 13 shows the units required for hemicellulose extraction and conversion to ethanol and acetic acid. They include wood extraction, acid hydrolysis of the extract using sulfuric acid for conversion of oligomeric carbohydrates into monomeric sugars and cleavage of LCC covalent bonds, filtration to remove precipitated lignin, liquid-liquid extraction followed by distillation to remove acetic acid and furfural from the sugar solution, liming to raise the pH to that required for fermentation of five and six-carbon sugars to ethanol, filtration of calcium sulfate, and finally distillation and upgrading the product to pure ethanol (99+%).

Design Basis

An existing Kraft pulp mill producing bleached mixed northeastern hardwood pulp was considered as the base case. For the IFBR modified plant, the pulp production was maintained constant with the added hemicellulose extraction process. Four pulp mill sizes were considered; a small plant producing 550 tonne per day representative of older bleached Kraft mills in the northeast, a 750 tonne per day pulp representative of a more modern mill, a large plant having a capacity of 1,000 tonne per day, and a state of the art 1,500 tonne per day Kraft mill. The simulation model for the modified Kraft pulp mill was developed primarily using the WinGEMS computer code. The energy balance calculations associated with distillation and liquid-liquid extraction were performed using ASPEN-Plus Software (*39*). The final brown-stock pulps were assumed to have a Kappa number of 17 and TAPPI viscosity of 37 centipoises.

Extraction Process

The extraction was performed in a separate continuous vessel prior to the continuous digester for pulp production using 1% total titratable alkali based on wood (*40*). Extraction conditions, pulp yield, extract composition and pulp physical properties were similar to those determined in laboratory experiments [Genco, (*30*)]. Nine percent (9%) of the wood was assumed to be removed by the extraction process. An anthraquinone charge of 0.05% (on wood) was included in the green liquor as a catalyst to maintain pulp yield. Wood chips are pre-steamed in a steaming vessel, then heated to the extraction temperature of 160 °C in 50 minutes and extracted for 110 minutes at 160 °C. This gave a total H-factor of 750 hours during the cooking process. The liquid containing extracted hemicellulose and lignin was separated from the chips, and the chips are conveyed into the pulping digester.

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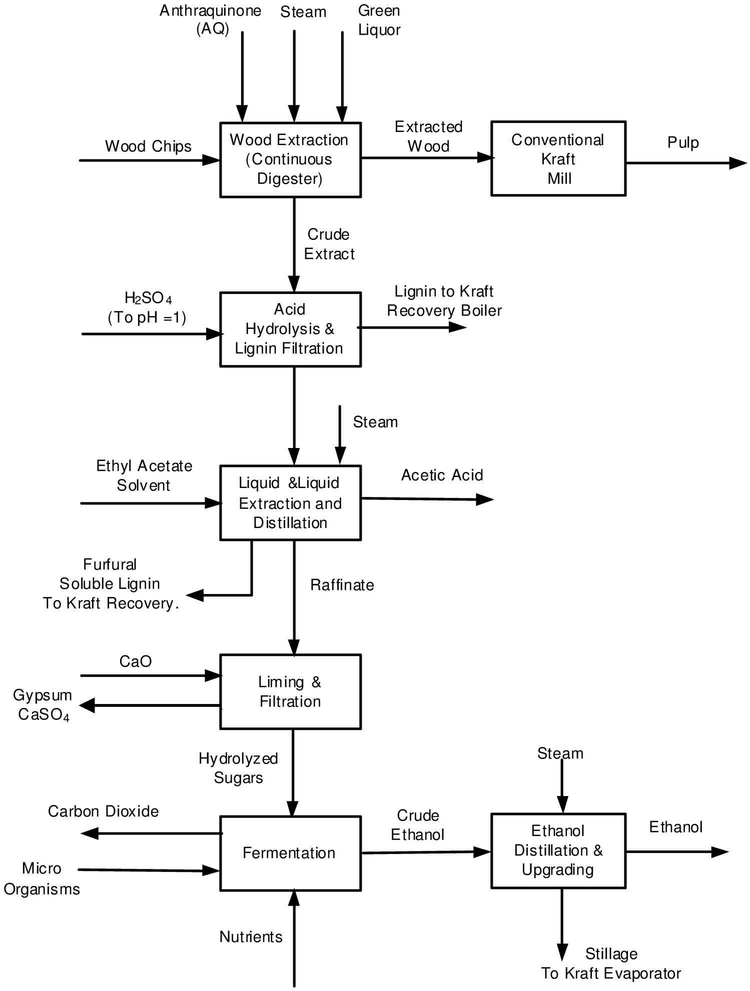
Anthraquinone (AQ)
Steam
Green Liquor
Wood Chips
Wood Extraction (Continuous Digester)
Extracted Wood
Conventional Kraft Mill
Pulp
Crude Extract
H_2SO_4 (To pH =1)
Acid Hydrolysis & Lignin Filtration
Lignin to Kraft Recovery Boiler
Steam
Ethyl Acetate Solvent
Liquid &Liquid Extraction and Distillation
Acetic Acid
Furfural Soluble Lignin To Kraft Recovery.
Raffinate
CaO
Gypsum $CaSO_4$
Liming & Filtration
Hydrolyzed Sugars
Steam
Carbon Dioxide
Micro Organisms
Fermentation
Crude Ethanol
Ethanol Distillation & Upgrading
Ethanol
Nutrients
Stillage To Kraft Evaporator

Figure 13. Modified kraft pulp mill for the production of acetic acid and ethanol.

Alkaline Pulping Conditions

The pulping conditions were assumed to be 14% EA in the pulping portion of the cook at a total H-factor of 1,350 hours including approximately 750 hours in the extraction vessel. The pulp yield was taken to be 47% and was the same for the base case. The Kraft control was assumed to use 15% EA, 30% sulfidity and H-factor of about 1,350 hours.

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Acid Hydrolysis and Lignin Removal

The hydrolysis of carbohydrates was assumed to follow the kinetics specified by Garrote et al. (*41*) for sulfuric acid hydrolysis of an Eucalyptus extract. Complete hydrolysis was shown to occur at a pH of 1.0 at 126°C. This required addition of sulfuric acid to lower the pH, and conversion of acetate to acetic acid by protonation. A hydrolysis time of 1.3 hours was needed for complete hydrolysis of the xylan oligomers. At these conditions it was assumed that all lignin is precipitated and can be separated from the liquor using pressure filtration. This assumption has been verified experimentally.

Acetic Acid and Furfural Recovery

Following lignin separation a liquid- liquid extraction process was used to extract acetic acid and furfural from the liquor. Seader and Henley (*42*) discuss the recovery of acetic acid from water using liquid-liquid extraction with ethylacetate as solvent, which is used commercially (http://www.ddpsinc.com/ProcessProfiles/pp50.html). Process simulation studies were conducted using guaiacol as a model compound for soluble lignin. The extracted acetic acid and furfural can be further purified by distillation (not shown) and are sold as final products at 99+% purity.

Liming and Gypsum Removal

After liquid-liquid extraction, calcium oxide (CaO) is used to "lime" the liquid. The main function is to adjust the pH to near neutral for ethanol fermentation. The lime also acts as a bactericide. The calcium precipitates the sulfate ions as gypsum [$CaSO_4$•($2H_2O$] to a concentration level which can be tolerated by the fermentation culture. Gypsum is removed using plate and frame filtration.

Fermentation and Ethanol Recovery

The micro-organism *E. coli* (KO11) was used to ferment C5 and C6 sugars simultaneously to ethanol (*43–45*). *E. coli* was found to be tolerant to low levels of soluble lignin [Walton, (*46*)]. The efficiency of converting C5 and C6 sugars into ethanol was set at 90% of the theoretical yield based upon the work of Wooley et al (*43*). Ethanol was processed after fermentation by pre-distillation to 50% purity and then further distillation to 95%, the azeotrope concentration. The ethanol is then further concentrated to 99.9% by using molecular sieve technology. Stillage from ethanol purification and upgrading is returned to the kraft recovery process. (http://www.ethanolindia.net/molecular_sieves.html).

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Ethanol and Acetic Acid Production Rates

Equations for estimating the production rates (y, millions gallons per year) for ethanol and acetic acid have been developed,

$$y_i\,(Million\ Gallon\,/\,Yr) = K * P(Tonne\,/\,Day) \qquad (1)$$

where P is the Kraft production rate (P, Tonne/Day) and K is an empirical constant (K) that is listed in Table 7 for ethanol and acetic acid. These equations assume that the plant operates 350 days per year (96% capacity factor). For a 1,000 tonne/day pulp mill, the ethanol production rate for would be approximately 31.6 tonne/day (3.7 million gallons/year) and 50.0 tonne/day of acetic acid (4.4 million gallons/year).

Energy Consumption

The addition of the hemicellulose extraction process to a Kraft pulp mill reduces the energy that is obtained from the residual pulping liquor. The net energy output is illustrated in Figure 14 in terms of millions of BTU per hour as equivalent steam. The net energy output of the mill is plotted as a function of pulp mill size. On average, the modified Kraft mill would produce approximately 30 to 40% less steam than the conventional Kraft mill because about 10% of the wood mass is extracted and the energy content of this mass appears as the energy content of the ethanol and acetic acid. Also, additional steam is required primarily for distillation (*39*).

Economic Cases Considered

Four cases were considered depen*d*ing upon whether an extraction vessel was assumed to be available or not, and whether the utilities and wastewater treatment systems had to be upgraded to handle the additional processing.

Table 7. Coefficient (K) for production equations

Chemical (i)	***(K)***
Ethanol	3.7×10^{-3}
Acetic Acid	4.4×10^{-3}

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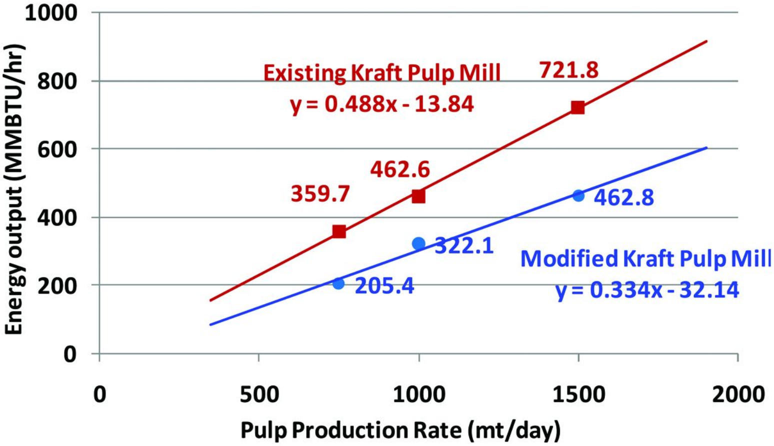

Figure 14. Energy output versus plant size for kraft mill using mixed northeastern hardwood chips.

Capital and Yearly Manufacturing Costs

Capital and operating cost for the process were estimated by methods presented by Turton and co-workers (*47*) and Peters and Timmerhaus (*48*). The capital cost estimates were based on information provided by Mitchell (*49*) and Mao (*40*). The total installed equipment cost of the unit processes in the wood to ethanol plant was obtained by multiplying the cost of purchased equipment by an installation factor; which amounted to about 3.55. In making the capital cost analysis reported here, 20% of the cost of a new continuous digester was applied to the project for modification of the impregnation vessel and/or connecting two continuous digesters in series. For those cases requiring a new extraction vessel, the full cost was applied.

The cost of manufacture or yearly operating cost was estimated from five basic costs, each of which was estimated separately; (1) raw materials, (2) labor, (3) waste treatment, (4) utilities and (5) capital charges. All other costs were applied as factors associated with these basic costs. The raw materials, utility requirements and waste material produced were determined from the WinGEMS and ASPEN plus computer simulation models and depend upon plant size. Unit costs for the raw materials, utilities, waste treatment and labor are summarized by Mao (*40*). A capital recovery factor of 16.5% was used to account for repayment of the capital, plus interest on borrowed money and local taxes.

The estimated capital and yearly operating costs for the four cases are summarized in Table 8. The economic analysis show quite clearly that the total capital cost will depend upon the plant size and the availability of an extraction vessel, and suitable of the existing utilities and waste treatment system. It is apparent that the cases with the highest capital investment are those that require the purchase and installation of a new extraction vessel and where the waste water treatment and utilities need to be upgraded. Because of capital charges and

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ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

the need to repay the investment cost, it is clear that the project with high capital investment invariable lead to high annual operating cost.

Biorefinery Revenues

The selling price of ethanol and food grade acetic acid was taken to be \$2.00 and \$4.00 dollars per gallon respectively. Revenues from the process are summarized in Table 9 and will vary with the volume of products produced and the selling prices. A revenue penalty was applied to the revenue stream to account for steam reductions in steam available for export and electrical energy from the modified Kraft mill. No penalty was assessed for any possible loss of pulp yield loss.

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Table 8. Estimated capital (millions \$) and annual cost (millions \$/year)

Pulp Rate (Tonne/ Day)	*Case 1 Vessel Available, Upgraded Utilities*		*Case 2 Vessel Available, Utilities Available*		*Case 3 New Vessel, Upgraded Utilities*		*Case 4 New Vessel, Available Utilities*	
	Cap cost 10^6\$	***Annual 10^6\$/Y***	***Cap cost 10^6\$***	***Annual 10^6\$/Y***	***Cap cost 10^6\$***	***Annual 10^6\$/Y***	***Cap cost 10^6\$***	***Annual 10^6\$/Y***
550	23.1	10.8	18.2	9.9	39.1	13.7	34.2	12.2
750	28.6	13	22.7	11.9	48.1	16.5	42.2	15.4
1000	34.9	15.6	27.8	14.4	58.5	19.8	51.4	18.5
1500	46.2	20.6	37.1	18.9	76.9	26.1	67.8	24.4

Table 9. Revenues to biorefinery for northeastern mixed hardwood

Mill Size (Tonne/day)	***Million Dollars/year***			
	Acetic Acid	***Ethanol***	***Lost Steam***	***Total***
550	9.7	4.1	-1.89	11.88
750	13.2	5.6	-2.58	16.19
1000	17.6	7.4	-3.44	21.59
1500	26.4	11.1	-5.17	32.39

Unit Production Cost

The unit production costs for the 1,000 tonne/day pulp mill case are summarized in Table 10. The unit production costs were estimated from the operating cost by assigning a portion of the operating cost to the production of both ethanol and acetic acid. The production costs for ethanol varied between $1.63 and $2.07/gallon depending upon the extraction vessel being available and whether the waste treatment system and utilities needed to be upgraded. Similarly for acetic acid the unit production cost varied between about $1.98 and $2.75 per gallon.

Table 11 lists the unit production cost as a function of plant size for Case 2, extraction vessel available and on upgrading of utilities required, which is the most economic case. The production cost of ethanol varied between $1.32 to $1.89 per gallon; while for acetic acid it varied between $1.75 and $2.51 per gallon. These estimates vary strongly as a function of size. Huang and co-workers (*15*) estimate the minimum selling price for ethanol produced in a Kraft mill biorefinery at $3.41/gallon that uses 2,000 dry tonne per day of wood. Huang postulates that the $3.41/gallon price can be reduced to $1.86/gallon if 56% more ethanol is produced by converting the cellulose in the short fiber fraction of the pulp into ethanol.

Table 10. Unit production cost 1,000 tonne per day pulp mill)

Case (1000 Tonne/Day Pulp Mill)	*Ethanol ($/gallon)*	*Acetic Acid ($/gallon)*
Case 1. Vessel Available with Upgrading	1.63	2.16
Case 2..Vessel Available and No Upgrading	1.49	1.98
Case 3. New Vessel With Upgrading	2.07	2.75
Case 4. New Vessel and No Upgrading	1.94	2.57

Table 11. Unit production cost as a function of size for Case 2 (vessel available and no utilities upgrading)

Pulp Mill Size (Tonne per Day)	*Ethanol ($/gallon)*	*Acetic Acid ($/gallon)*
550	1.89	2.51
750	1.66	2.21
1000	1.49	1.98
1500	1.32	1.75

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Discounted Cash Flow Analysis

A profitability analysis was applied using the discounted cash flow rate of return on investment method. In this method the interest rate (i) is found, so that the negative cash flows to the project are just balanced by positive cash flows (Mao (*18*)). The analysis results are summarized in Figure 15. In the discounted cash flow rate of return method, a project is judged to be a good, neutral or poor by comparing the calculated discounted rate of return (i) to the cost of capital (i_C), which in the present study was taken to be 10%. The cost of capital (i_C) is the minimum rate of return that is considered acceptable assuming a low risk on the enterprise. Similarly at 0% rate or return, the positive cash flows to the investment just equal the negative cash flows to the project. It is clear from Figure 15 that the larger the plant size, the more profitable the investment. The condition leading to the highest discounted cash flow rate of return is Case 2, which minimizes capital investment. In Kraft pulp mills where these conditions are fulfilled, the rate of return can be as high as 18% for the 1,500 tonne/day case. For Case 2, the cost of capital (10%) is reached for a 900 tonne/day pulp mill. Under no conditions was it found to be profitable to install the hemicellulose extraction process where a new extraction vessel was required. As a general rule, as the capital investment increases, the discounted cash flow rate of return on investment decreased. For the cases where a new vessel is required, the discounted cash flow rate of return is invariable negative except for very large pulp mill sizes. As further work it is recommended to perform a sensitivity analysis of the price of ethanol and acetic acid on the profitability of the NNGL process.

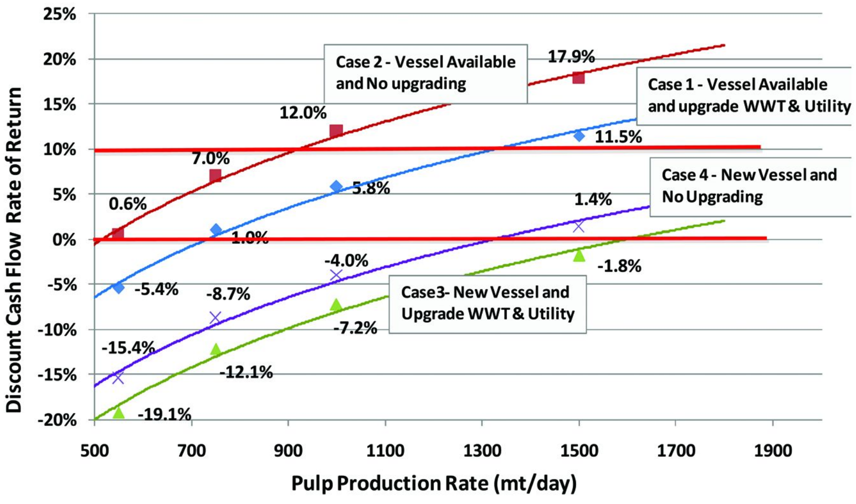

Figure 15. Discounted cash flow rate of return for different cases.

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Deployment of 'Near-Neutral' Extraction Technology

The original concept (*50*) of the IFPR has become the foundation of the University of Maine Forest Biorefinery framework that was the basis of a project proposal to the US DOE for an Integrated Biorefinery in Old Town, Maine. The IFPR is based on 'near-neutral' pre-pulping extraction of hemicelluloses from wood chips while preserving cellulose fibers for pulp production. In late 2007, the Old Town mill modified its pulping system to incorporate UMaine's pre-pulping hemicellulose extraction technology, while researchers at UMaine successfully demonstrated conversion of wood-extracts into ethanol. This was instrumental in making it possible for the Old Town pulp mill to be selected for a $30 million competitive award from the U.S. DOE in April 2008, for a small scale commercial technology demonstration of an integrated biorefinery for coproduction of pulp and biofuels. Since November 2008 under new ownership (Red Shield Acquisitions LLC, RSA), the pulp mill has undertaken an aggressive mill conversion program to develop the Old Town Fuel & Fiber (OTFF) facility. Beginning in Summer 2009, OTFF has conducted several pre-pulping extraction trials using UMaine's near neutral hemicellulose extraction technology at commercial scale.

The Old Town pulp mill has converted its two existing single-vessel digester systems to one, two-vessel pulping system. The existing chip feedline is used to feed woodchips to the second vessel which is now used as a vapor-phase impregnation vessel (IV) for extraction (see Figure 16). Partially cooked impregnated chips are transferred with a new bottom circulation loop from the second vessel (K2) to the existing first vessel which is used as the main cooking vessel (K1) i.e. kraft digester. The aqueous extract is taken out of the impregnation vessel (K2) during the trials to be used for further conversion to biofuels, the remainder is sent to the black liquor recovery systems at the present time. Brownstock from K1 goes to washing and screening as usual.

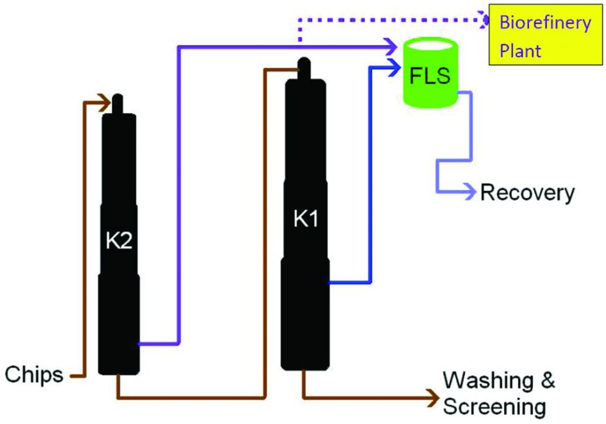

Figure 16. Old Town pulp mill two-vessel system for deployment of 'near-neutral' extraction technology.

The new two-vessel system has been running well now for more than two years. Commercial scale extract production, and pilot scale extract characterization and conversion studies provide data necessary to obtain design parameters required for the proposed biofuel co-production plant. Several million gallons of extract have been produced during trials while maintaining quality market pulp output, over more than 300 hours of trial experience. Design of the biorefinery plant to convert pre-pulping wood extract into biobutanol is underway in 2010.

Conclusion

Part of the hemicelluloses may be extracted from hardwood chips with small effect on yield and properties of fibers produced by subsequent alkaline pulping when the pH of the final extract is “near-neutral”. This is achieved by adding a limited amount of alkali, such as green liquor or Na_2CO_3, to the aqueous extract at 140-160 °C for about 2 hours at L/W ratio of 3-4 L/kg. The treatment is called the “near-neutral” pre-extraction process. The hemicelluloses dissolve in the form of oligomers (except for arabinose and galactose which are mostly monomeric), degraded sugars and sodium acetate/acetic acid. The total sugar concentration of the extract after acid hydrolysis decreases strongly with increasing green liquor charge. The total sugar concentration in the extract derived from northeastern hardwood chips decreases from about 30 g/L after pure water extraction at final extract pH of 3.3-3.4, to about 10 g/L at final pH of 4.8 with 2% GL charge, to 6 g/L at final pH of 5.6 with 4% GL charge. At 2 or 4% GL the total kraft pulp yield is 3 or 1.5% lower respectively than the control kraft pulp obtained without pre-extraction. With pure water extraction the final kraft pulp yield is 12% lower than the control. Thus the use of green liquor minimizes the total pulp yield loss at the expense of significantly less recoverable sugars in the extract compared to pure water extraction; which however suffers from to an unacceptable pulp yield loss. Another major practical advantage of “near-neutral” green liquor extraction is that the digester remains free of sticky lignin for extracts of pH ≥ 5.0. At autohydrolysis conditions, with a final pH of 3-4, the formation of lignin-based precipitates are the principal reason why hemicelluloses are presently not recovered during the commercial prehydrolysis-kraft process. The amount of dissolved oligomeric sugars during the “near-neutral” process is not only controlled by the extract, but also by the solubility of the oligomers. Thus if the solution for dissolution is larger (i.e. L/W is larger), then more sugars may be removed from wood, and vice versa. At about pH 5 or higher all dissolved sugar oligomers are deacetylated. However in a pure water extract of pH 3-4 about half of the removed acetyl groups are still attached to the dissolved oligomers while about half is cleaved off. Minimum deacetylation of wood occurs at pH 4 near the pKa of acetic acid when the sum of alkaline and acid catalysed hydrolysis rate of the acetyl groups is at the lowest level. The concentration of acetate/acetic acid in the extract is higher for mixed northeastern hardwoods than southern hardwoods because the former contains more acetyl groups (3.65 vs 2.76 %).

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The production cost for ethanol is estimated to be between $1.32 and $2.07/ gallon; and for acetic acid between $1.75 and $2.75 per gallon depending upon the mill size and the equipment available for use with the new process. The unit production cost decreases significantly as the plant size increases and as the capital investment is minimized. Installing a new extraction vessel reduces the discounted cash flow rates of return on investment considerably. Since only a portion of the hemicelluloses in the wood are extracted, the amount of ethanol and acetic acid are relatively small by industrial standards; but could potentially represent positive revenue for a kraft pulp mill operator. For a biorefinery concept based on producing ethanol and acetic acid at an existing kraft mills, the processing must become simpler to reduce the capital expenditures. A reduction in capital expense will reduce annual operating cost and improve the discounted cash flow rate of return. The current estimated rates of return are unacceptably low except for very large mills that have an existing extraction vessel that can be modified appropriately and have sufficient utilities and waste treatment facilities exist to accommodate the additional processing.

The near-neutral green liquor hemicellulose extraction technology for coproduction of pulp and biofuels is being demonstrated in a kraft pulp mill in Old Town, Maine by Red Shield Acquisitions LLC, RSA) in collaboration with the University of Maine.

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References

1. Roberts, D. Future Bio-Pathways Project, Forest Products Association of Canada, February 2010.
2. Retsina, T.; PylkkanenV. Converting Pulp Mills to Biorefinery – A Comparison of Technologies, 94th Annual Meeting, PAPTAC, Montreal, QC, February 2008.
3. Demirbas, A. *Energy Sources* **2005**, *27*, 761–767.
4. Paredes, J. J.; Jara, R.; van Heiningen, A.; Shaler, S. M. *For. Prod. J.* **2008**, *58* (12), 56–62.
5. Brasch, D. J.; Free, K. W. *Tappi* **1965**, *48* (4), 245–248.
6. Casebier, L. C.; Hamilton, J. K. *Tappi* **1969**, *52* (12), 2369–2377.
7. Tunc, M. S.; van Heiningen, A. R. P. *Holzforschung* **2008**, *62*, 539–545.
8. Amidon, T.; Liu, S. *Biotechnol. Adv.* **2009**, *27* (5), 542–550.
9. Mendes, C. V. T.; Carvalho, M. G. V. S.; Baptista, C. M. S. G.; Rocha, J. M. S.; Soares, B. I. G.; Sousa, G. D. A. *Food Bioprod. Process.* **2009**, *87*, 197–207.
10. Yoon, S.-H.; MacEwan, K.; van Heiningen, A. *Tappi J.* **2008**, *7* (6), 27–31.

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11. Al-Dajani, W. W.; Tschirner, U. W.; Jensen, T. *Tappi J.* **2009**, *8* (9), 30–37.
12. Yoon, S. H.; van Heiningen, A. R. P. *Tappi J.* **2008**, *7*, 22–27.
13. Frederick, W. J.; Lien, S. L.; Courchene, C. E.; DeMartini, N. A.; Ragauskas, A. J.; Iisa, K. *Biomass Bioenergy* **2008**, *32* (12), 1293–1302.
14. Al-Dajani, W. W.; Tschirner, U. W. *Tappi J.* **2008**, *7* (6), 3–8.
15. Huang, H. J.; Ramaswamy, S.; Al-Dajani, W. W.; Tschirner, U. W. *Bioresource Technol.* **2010**, *101*, 624–631.
16. Mao, H.; Genco, J. M.; Yoon, S.-H.; van Heiningen, A.; Pendse, H. *J. Biobased Mater. Bioenergy* **2008**, *2*, 177–185.
17. Yoon, S.-H.; van Heiningen, A. *J. Ind. Eng. Chem.* **2010**, *16* (1), 74–80.
18. Mao, H.; Genco, J. M.; van Heiningen, A.; Pendse, H. *BioResources* **2010**, *5* (2), 525–544.
19. Kautto, J.; Henricson, K.; Sixta, H.; Trogen, M.; Alén, R. *Nord. Pulp Pap. Res. J.* **2010**, *25* (2), 233–242.
20. Iakovlev, M.; van Heiningen, A.; Pylkkänen, V. SO_2−Ethanol−Water Pulping of Softwoods, The 6th Johan Gullichsen Colloquium, Finnish Paper Engineers' Association, Espoo, Finland, November 15, 2007, pp 39−45.
21. Horhammer, H.; Walton, S.; van Heiningen, A. Producing Lactic Acid from Larch in a Kraft Mill, Proceedings of the 7th Biennial Johan Gullichsen Colloquium − Forest Biomass − Quo Vadis?, Espoo, Finland, November 19, 2009, pp 67−76.
22. Jin, Y.; Jameel, H; Chang, H.; Phillips, R. *J. Wood Chem. Technol.* **2010**, *30* (1), 86–104.
23. Wu, S.-F.; Treasure, T.; Jameel, H.; Chang, H.-M.; Phillips, R. Technical and Economical Feasibility of Repurposing Uneconomic Softwood Kraft Pulp Mills to Ethanol Production, PAPTAC 96th Annual Meeting, Montreal, Canada, 2010, pp 70−78.
24. Yoon, S.-H.; Tunc, M. S.; van Heiningen, A. *Tappi J.* **2011**, *10* (1), 7–15.
25. van Heiningen, A. Integrated Forest Products Refinery (IFPR), DOE Final Report, 2010. http://www.osti.gov/bridge/servlets/purl/979929-FuFfqu/979929.pdf.
26. Genco, J. M; Busayasakul, N.; Medhora, H. K.; Robbins, W. *Tappi J.* **1990**, *73* (4), 223–233.
27. Bose, S. K.; Barber, V. A.; Alves, E. F.; Kiemle, D. J.; Stipanovic, A. J.; Francis, R. C. *Carbohydr. Polym.* **2009**, *78*, 396–401.
28. Tunc, M. S.; Lawoko, M.; van Heiningen, A. *BioResources* **2010**, *5* (1), 356–371.
29. Walton, S.; Hutto, D.; Genco, J.; van Walsum, P.; van Heiningen, A. *Ind. Eng. Chem. Res.* **2010**, *49* (24), 12638–12645.
30. Genco, J. M.; Mao, H.; van Heiningen, A.; Zou, H.; Luo, J.; Pendse, H. Technical Economic Evaluation of a Northern Hardwood Biorefinery using the "Near-Neutral" Hemicellulose Pre-Extraction Process, Eng., Pulping and Environmental Conference, Portland, OR, August 24−27, 2008.
31. Leschinsky, M.; Sixta, H.; Patt, R. *BioResources* **2009**, *4* (2), 687–703.
32. Luo, J. M.S Thesis, University of Maine, Orono, ME, 2010.

33. Vuorinen, T. Chemistry of Pulping and Bleaching, Lecture notes; Department of Forest Products Technology, Helsinki University of Technology: Espoo, Finland, 2005.
34. Sjöström, E. Wood Chemistry Fundamentals and Applications, 2nd ed.; Academy Press, Inc.: San Diego, 1993.
35. Scott, R. W. *Anal. Chemistry* **1979**, *51* (7), 936–941.
36. Sundberg, A.; Sundberg, K.; Lillandt, C.; Holmbom, B. *Nord. Pulp Pap. Res. J.* **1996**, *11* (4), 216.
37. Chen, X.; van Heiningen, A. *Bioresource Technol.* **2010**, *101* (20), 7812–7819.
38. Leschinsky, M.; Patt, R.; Sixta, H. Water Prehydrolysis of *E. Globulus* with the Main Emphasis on the Formation of Insoluble Components, PulPaper 2007, Helsinki, Finland, June 5−7, 2007.
39. Mao, H. Master's Thesis, University of Maine, Orono, ME, 2007.
40. Smook, G. A. Handbook for Pulp and Paper Technologists, 3rd ed.; Angus Wilde Publications: Vancouver, B.C., 2002; pp 75−84.
41. Garrote, G.; Dominguez, H.; Parajo, J. C. *Bioresource Technol.* **2001**, *79*, 155–164.
42. Seader, J. D.; Henley, E. J. *Separation Process Principles*; 2nd ed.; John Wiley: New York, 2006; p 296.
43. Wooley, R., et al. NREL/TP-580-26157,1999.
44. Aden, A.; Ruth, M.; lbsen, K. NREL report, NREL/TP-510-32438;2002: pp 7−36.
45. Amartey, S.; Jeffries, T. *World J. Microbiol. Biotechnol.* **1996**, *12*, 281–283.
46. Walton, S. Ph.D. Thesis, University of Maine, Orono, ME, 2009.
47. Turton, R., et al. *Analysis, Synthesis, and Design of Chemical Processes*; Prentice Hall: Upper Saddle River, N.J., 2003.
48. Peters, M. S.; K. D., Timmerhaus *Plant Design and Economics for Chemical Engineers*; McGraw Hill: New York, 1991.
49. Mitchell, J., M.S. Thesis, University of Maine, Orono, ME, 2006.
50. Van Heiningen, A. *Pulp Pap. Can.* **2006**, *107* (6), 38–43.

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Chapter 18

Integrated Forest Biorefinery – Prehydrolysis/Dissolving Pulping Process

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The hemicelluloses removal is a key step of the dissolving pulp production process, which is carried out in a steam or water pre-hydrolysis stage. The conversion of hemicelluloses to other value added products was comprehensively described in the literature as a part of forest biorefinery utilization. Principally, the hydrolysis stage of a dissolving pulp production process can be the first step of producing value added products, which converts the pulp mill to a forest biorefinery unit. In this chapter, the impacts of hydrolysis and process conditions on biomass removal from wood chips are discussed, and the characteristics of industrially produced hydrolysis liquor (PHL) are evaluated. Additionally, the biorefinery processes and units, which are compatible with the existing configuration of kraft-based dissolving pulp production process, and the potential value added products and pathways are discussed.

Introduction

Traditionally, pulp is the main product of pulp mills, which is used for the production of various paper grades. However, the rising cost of energy and the tight competition from low-cost countries using fast growing raw materials adversely affected the North American pulp and paper business (*1*, *2*). To increase the revenue, the North American mills are seeking for new products, new strategies, and new business areas. The application of forest biorefinery concept to the North American mills has been proposed as a promising approach to overcome this difficulty (*3–5*). One key criteria of the forest biorefinery

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utilization is its adoptability. The established infrastructure of North American mills, well-trained workforce, strong community support, and abundant wood fiber supplies provide unique opportunity to adopt forest biorefinery processes along with the replacement or as the replacement in these mills (*5*, *6*).

Woody materials consist of hemicelluloses, cellulose, lignin, and small amount of extractives. By adopting the forest biorefinery concept, several value added products can be produced in pulp mills. It was proposed that hemicelluloses can be used as the raw materials for furfural, hydroxymethylfurfural (HMF), xylitol, ethanol, and lactic acid productions (*7–10*), or as strength additives for papermaking (*12*). The lignin of woody materials has a variety of industrial applications, e.g., a fuel source (*1*, *9*, *13–17*), dispersing agent, emulsion stabilizer, or rheology control (*18*). It can also be used as a raw material for value added products, e.g., phenols, carbon fibers, binder resins, soil improvements (*1*, *5*, *9*, *19*), polyurethanes, plastics/polymers (*20–22*) or for biocomposites (*23*).

Dissolving pulp accounts for 2.5% of the total pulp production, among which 40% is produced based on pre-hydrolysis kraft process (*24*). The hemicelluloses removal is a critical step in dissolving pulp production process (*13*, *25*). Several processes have been proposed for converting kraft pulping process into a forest biorefinery unit (*1*, *5*, *14*, *26–28*). In the literature, the conversion of hemicelluloses to value added by-products were described (*12*, *29–31*). Naturally, the pre-hydrolysis kraft dissolving pulp production process fits into the forest biorefinery concept. In this chapter, the impacts of hydrolysis and process conditions on the biomass removal from wood chips are discussed. Additionally, the characteristics of pre-hydrolysis liquor are systematically discussed. Procedures to recover the lignocellulosic materials dissolved in pre-hydrolysis liquor and convert them to various by-products along with their advantages and disadvantages are discussed. For simplicity, the pre-hydrolysis liquor is noted as PHL in this chapter.

Dissolving Pulp Production Process Based on Pre-Hydrolysis Kraft Process

There are three major sections in the production of a dissolving pulp process based on the pre-hydrolysis kraft process: hydrolysis, pulping, and bleaching. Figure 1 shows the block diagram for this process. In the hydrolysis step, the majority of hemicelluloses and a part of lignin are removed from the wood chips. Then, the conventional kraft pulping is conducted on the pre-hydrolyzed wood chips for removing the majority of lignin present in the wood chips. During the pulping, remaining hemicelluloses and a part of cellulose are also dissolved. The fundamental and process concepts of kraft pulping and their influences on the pulp properties are well documented in the literature (*25*, *32*). After kraft pulping, the residual lignin is removed from the pulp by using bleaching chemicals in subsequent bleaching stages, where a part of cellulose is also dissolved and removed from the unbleached pulp.

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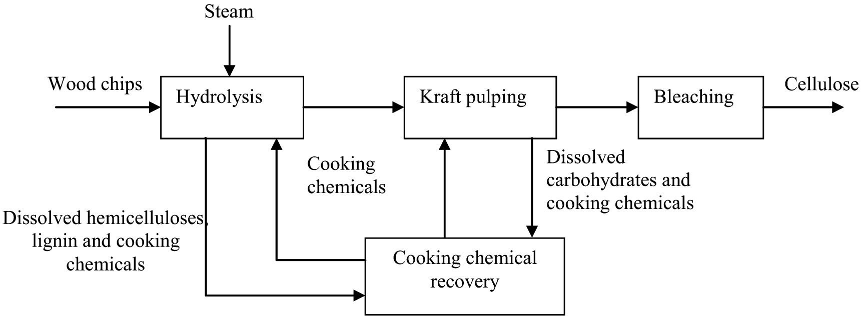
Steam
Wood chips
Hydrolysis
Kraft pulping
Bleaching
Cellulose
Cooking chemicals
Dissolved carbohydrates and cooking chemicals
Dissolved hemicelluloses, lignin and cooking chemicals
Cooking chemical recovery

Figure 1. Block diagram of dissolving pulp production based on the pre-hydrolysis kraft process. (Reproduced with permission from reference (25). Copyright 2006 Wiley VCH).

Hydrolysis Methods

The hydrolysis stage of dissolving pulp production process can be performed in aqueous or steam phase in pilot scale or industrial scales, respectively (*13*, *25*, *33*). The aqueous phase extraction is conducted using hot water at an elevated temperature (140-170 ºC) (*15*, *28*, *34*), which dissolves the hemicelluloses from the wood chips. This process also results in structural modification and dissolution of lignin: 1) The hot water extraction causes a fast depolymerization of lignin and the cleavage of lignin-carbohydrate bonds; 2) lignin repolymerization is occurred in a slow rate in the presence of organic acid formed in the pre-hydrolysis liquor (PHL) (*13*). The lignin presence would affect the further processability and utilization of the PHL in the production of value-added products from the dissolved sugars. Other issues of the hot water extraction may include the precipitation of lignin compounds on pulps that would negatively affect the process operation. Consequently, the hot water extraction has been limited to laboratory and pilot scale studies (*13*). The steam hydrolysis has been a well established process for removing hemicelluloses prior to kraft pulping in a dissolving pulp production process (*13*, *25*, *33*).

Commercial Technology for Hydrolysis Process

The dissolving pulp production process based on the pre-hydrolysis kraft process has been commercialized as Visbatch® and VisCBC operations in industry (*25*). In these systems, the hydrolysis is performed using saturated steam, preferably at a temperature of 170 ºC. Depending on the cellulose purity desired, the hydrolysis intensity is adjusted to a P-factor ranging from 300 to 1000 (*25*). After the hydrolysis, a neutralization step is required for extracting, degrading and removing the cleaved hemicelluloses from the wood chips, which is succeeded by a hot displacement (Visbatch®) or cooking stage (VisCBC) (*25*). Table I lists the mass balance of the most important wood components of *Eucalyptus urograndis* at a P- and H-factor of 300 via Visbatch® process (*25*). Evidently,

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most of xylan was removed in the pre-hydrolysis and neutralization stages. In the hot displacement stage, the neutralization liquor is displaced by a mixture of hot black liquor and white liquor. Most of lignin was removed in neutralization and hot displacement steps of the Visbatch® process (see Table I) (*25*). Subsequent kraft cooking is then conducted to produce pulp with a kappa number ranging from 45 to 10. After cooking, the cold displacement is performed (*25*). As can also be seen, the xylan, lignin, and cellulose contents of unbleached pulp dropped by 92%, 97.9% and 20.9% via the Visbatch® process.

Therefore, the hydrolysis step is a major stage of the dissolving pulp process since the removal of hemicelluloses mainly occurs in this step. In fact, the downstream pulping and bleaching processes are not sufficiently selective in removing hemicelluloses from the pulp. Therefore, the hydrolysis step directly impacts the quality of the final dissolving pulp (*13*, *25*, *33*).

Hydrolysis Intensity

The conditions of hydrolysis significantly affect the extraction of hemicelluloses from the wood chips. Although the concept of H-factor can be still applied for analyzing the intensity of hydrolysis, P-factor was specifically introduced for the hydrolysis stage to serve for such a purpose. Similar to H-factor, P-factor relates the temperature and time of hydrolysis by considering the activation energy for cleavage of glycosidic bonds for carbohydrate materials of wood chips. The detailed concept of P-factor is comprehensively illustrated in the literature (*25*, *28*). The simplified form of P-factor is presented in Equation 1 (*28*):

$$\text{P-factor} = \int_0^t \frac{k(T)}{k_{100°C}}\,dt = \int_0^t e^{40.48 - \frac{15106}{T}}\,dt \qquad (1)$$

The longer the time, or the higher the temperature of hydrolysis, the greater the intensity of hydrolysis, thus the higher the P-factor. Figure 2 shows the relationship between P-factor and the extraction of materials from mixed southern hardwoods (*28*). As can be seen, at a P-factor of 1000, almost 25% of the wood components were extracted, regardless of the time or temperature of the hydrolysis pretreatment.

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Table I. Mass balance of the most important wood components of *Eucalyptus urograndis* at a P- and H-factor of 300 via Visbatch® process. (Reproduced with permission from reference (*25*). Copyright 2006 Wiley VCH)

Process steps	*Yield, %*	*Cellulose, %*	*Xylan, %*	*Total carbohydrates, %*	*Lignin, %*
Wood	100	45	15.8	70.5	28.7
Pre-hydrolysis	91.9	44.1	10.2	62	27.9
Neutralization	65.8	42.1	3.8	49.6	14.7
Hot displacement	44.2	38	1.4	41.2	2.8
Cooking	40.4	36.8	1.4	39.5	0.7
Cold displacement	38.9	36	1.3	38	0.7
Unbleached pulp	38.1	35.6	1.2	37.4	0.6

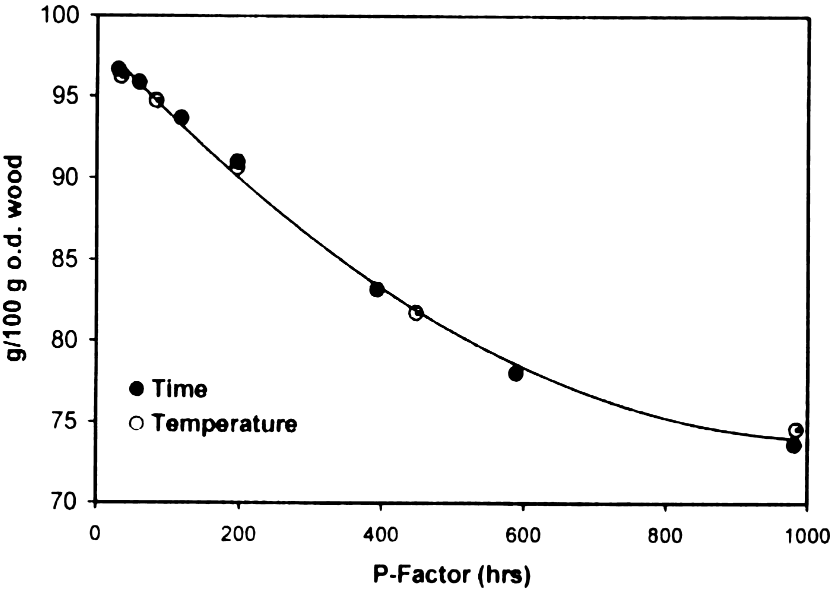

Figure 2. Yield of hot water hydrolysis of mixed southern hardwoods as a function of P-factor. (Reproduced with permission from reference (28)).

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Influence of Process Conditions on the Hydrolysis Efficiency

As described earlier, the PHL is mixed with cooking liquors at different stages in the VisCBC and Visbatch® processes. Although information is available on the effect of process conditions on the properties of dissolving pulp produced in kraft-based dissolving pulp process according to the VisCBC and Visbatch® techniques, very limited information is available on the properties of the PHL produced in these processes. This is partly due to the fact that the mixing of cooking liquors with the PHL results in high pH and temperature of the liquor. The hemicelluloses are oxidized and degraded in the PHL under these conditions (similar to the dissolved hemicelluloses in black liquor). Therefore, the mixed liquor after the hydrolysis stage would be difficult to be further utilized in the production of value-added products. Although the neutralization stage is very efficient on hemicelluloses removal from the wood chips (Table I), the extraction should be conducted by employing different approaches (rather than a neutralization step) so that the hemicelluloses were not degraded, if the PHL were to be utilized by following the forest biorefinery concept. Also, the PHL should be separated from the process prior to adding cooking liquors to the hydrolyzed wood chips. Prior to mixing with cooking liquors, the conditions of hydrolysis stage in the VisCBC or Visbatch® process and the properties of the PHL would be similar to those of hot water or steam hydrolysis conducted in laboratory and/or pilot scale practices.

On the other hand, compared with the steam hydrolysis, the hot water hydrolysis results in more hemicelluloses removal from the wood chips (*36*). That is why the research on the biorefinery concept and utilization of hot water hydrolysis has obtained great attentions.

The influences of process conditions on the properties of the PHL produced by hot water or steam hydrolysis are discussed in the following section, which provides a guideline for the hydrolysis stage/liquor of the VisCBC and Visbatch® techniques.

The optimum conditions for extracting hemicelluloses depend on wood spices. As the kinetics of hemicelluloses, lignin, and cellulose removals are different, the increase in the removal of one component does not necessarily imply the increase in the removal of the others from wood chips. Figure 3 shows the removals of different wood components during the hot water hydrolysis of mixed southern hardwoods as a function of P-factor (*28*). Evidently, almost 67% of hemicelluloses were removed at the P-factor of 1000, while the lignin and celluloses were only marginally removed.

Hot water or steam hydrolysis is an auto-catalytic process, in which the cleavage of acetyl and uronic acid substituent forms acetic and other organic acids. In other words, the liberation of acids from xylan of hardwood provides the acidity to catalyze the dissolution and depolymerization of remaining hemicelluloses from the wood chips (*28*, *34*, *35*). Figure 4 shows the formation of acetic acid and acetyl- containing dissolved organics during the hydrolysis of mixed hardwoods at 170 ºC (*34*).

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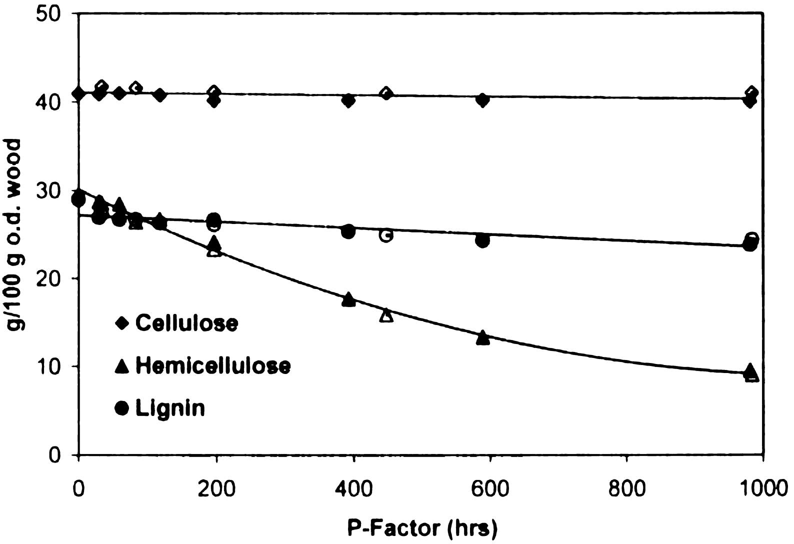

Figure 3. Wood components remaining in mixed southern hardwood after hot water hydrolysis as a function of P-factor. (Reproduced with permission from reference (28)).

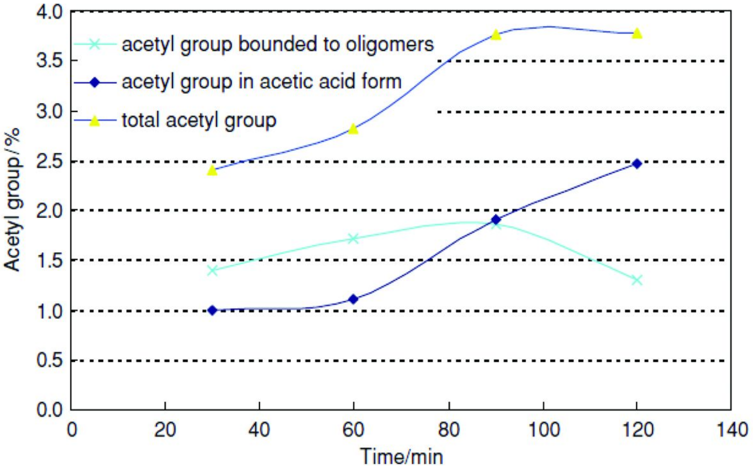

Figure 4. Acetyl group in acetic acid form and acetyl group bounded to oligomers in the hydrolysis of mixed hardwoods. (Reproduced with permission from reference (34). Copyright 2010 Wiley).

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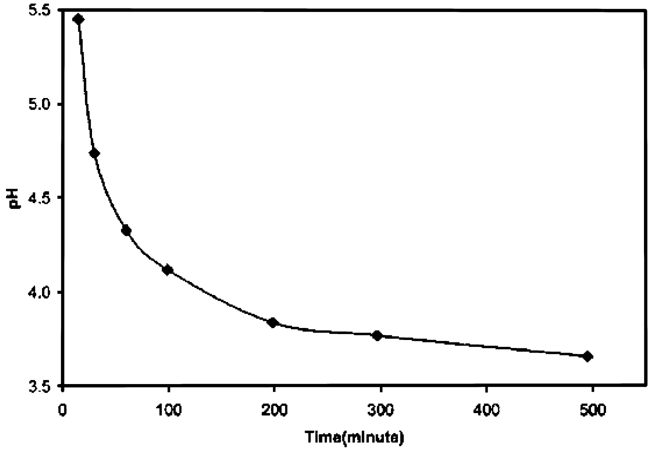

Figure 5. Changes in the pH of PHL during hydrolysis of mixed hardwood at 150 ºC. (Reproduced with permission from reference (15). Copyright 2008 ACS).

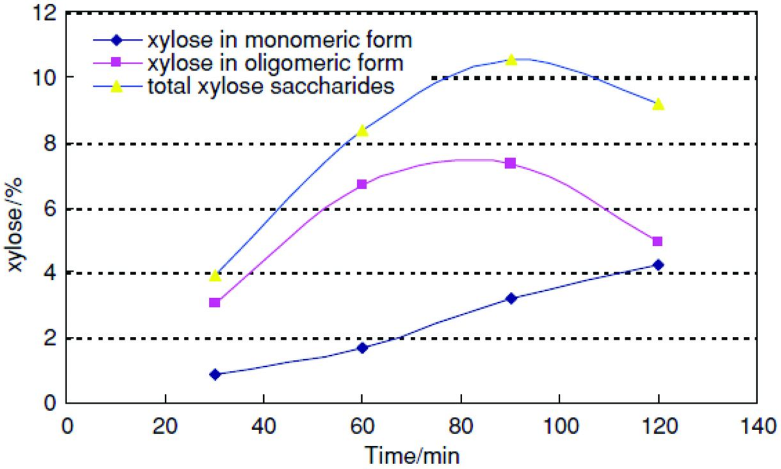

Figure 6. Formation of xylose/xylan in the hydrolysis of mixed hardwoods at 170 ºC versus the time of hydrolysis. (Reproduced with permission from reference (34). Copyright 2010 Wiley).

The formation of such acids decreases the pH of the liquor to 3-4, which facilitates the hydrolysis of polysaccharides to oligomeric and monomeric sugars (*35*). Figure 5 shows the final pH versus the extraction time for mixed hardwood at 150 ºC (*15*).

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Table II. Mass removal (g/l) (both monomers and oligomers) of mixed hardwood during the hot water hydrolysis under various conditions for 90 min. (Reproduced with permission from reference (*34*). Copyright 2010 Wiley)

Maximum temperature, ºC	*150*	*170*	*190*	*210*
Arabinose	0.3	0.3	0.11	0
Rhamanose	0.09	0.27	0.05	0.01
Galactose	0.13	0.46	0.29	0.52
Glucose	0.28	0.74	0.73	0.69
Xylose	0.98	8.34	3.92	0.05
Mannose	0.25	0.79	0.76	0.06
Acetylated groups	0.6	2.82	4.41	5.04
Furfural	0.01	0.37	2.99	3.65

However, by increasing the intensity of hydrolysis, hemicelluloses can be converted to furfural, which reduces the total amount of hemicelluloses in the PHL (*37*). Figure 6 shows the presence of xylose in monomeric and oligomeric forms versus the time of hydrolysis of mixed hardwoods, which was conducted at 170 ºC (*34*).

Table II lists the mass removal of mixed hardwood during the hot water hydrolysis under various conditions for 90 min. Evidently, the maximum hemicelluloses concentration was obtained at 170 ºC. However, the concentrations of furfural and acetic acid were increased by further increase of hydrolysis temperature at 210 ºC.

Therefore, to obtain the PHL with a high hemicelluloses concentration, an optimum condition should be determined, which depends on the wood species. The detailed hemicelluloses removal of aspen wood chips under various conditions is listed in Table III.

However, the dissolution of lignin is facilitated by the increase in the phenolic hydroxyl groups as a result of the cleavage of β-O-4 bonds, which hinders the quality of hydrolysis liquor for further hemicelluloses utilization (*13*, *33*). By increasing the hydrolysis intensity, the aliphatic hydroxyl groups and β-O-4 bonds decreased, while the phenolic hydroxyl group of lignin increased (*13*, *33*). It was reported that a high temperature and short reaction time are favorable for obtaining a high dissolved xylan yield (*37*).

Figure 7 shows the concentration of various hemicelluloses in the hydrolysis liquor of mixed hardwoods based on hot water extraction, and Figure 7 shows the removal rates of xylan and lignin from the wood chips versus time (*38*). As can be seen, the concentrations of hemicelluloses were the maximum after 40 min of

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hydrolysis, which was followed by the maximum xylan and lignin removal rates from the wood chips.

In the case of hot water hydrolysis, there are contradictory results in the literature about the influence of liquid-to-wood (L/W) ratio on the concentration of hemicelluloses in the PHL (*39*). In fact, liquid-to-wood ratio influenced the concentration of hemicelluloses of the PHL in some studies, whereas no effect was observed in other studies (*37*). A significant decrease in the xylan dissolution rate was observed on the hydrolysis of Eucalyptus saligna wood chips at 170 ºC when the L/W ratio was decreased from 5/1 to 2/1 (*37*). This decrease was probably due to the poor diffusion and impregnation of wood chips when decreasing the L/W (*39*). The wood chip size and the quality of chips also affected the hydrolysis efficiency (*37*).

Table III. Hemicelluloses removal of aspen wood chips in the hot water hydrolysis under different conditions. (Reproduced with permission from reference (*37*))

Conditions	*150 ºC, 4.5 h*	*160 ºC, 1.5 h*
Glucan	0.7	0.7
Xylan	8.9	8.7
Galactan	0.8	0.7
Arabinan	0.4	0.4
Mannan	0.7	0.6
Lignin	1.1	1.6
Others	6.4	5.7
Total	19	18.4

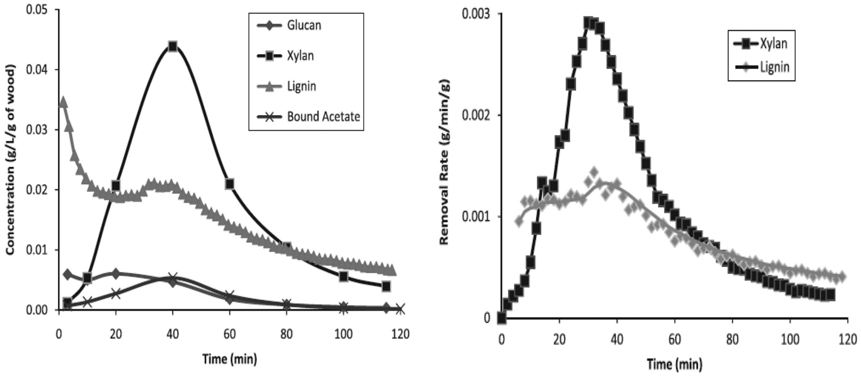

Figure 7. a) Concentration of hemicelluloses in the PHL, and b) removal rate of xylan and lignin during hot water extraction of mixed hardwood. (Reproduced with permission from reference (38). Copyright 2010 Elsevier).

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Characterization of Industrially Produced Pre-Hydrolysis Liquor (PHL)

As described previously, hemicelluloses, lignin and furfural have different end-use applications. Thus, the purity of the pre-hydrolysis liquor is of great importance, which influences the downstream processes and the type and quality of the value-added products obtained. The chemical composition of the PHL obtained from an industrially produced PHL based on steam hydrolysis is listed in Table IV (*17*). Evidently, the solid content of the PHL was approximately 5.5%, among which 1-1.4% was ash, 1-1.2% was lignin, 1.2% was acetic acid, and 1.5-1.7% was hemicelluloses.

The high ash content of the PHL is due to partly mixing of PHL with black liquor during the neutralization process prior to sampling, as described earlier. This analysis implies that hemicelluloses, lignin, and acetic acid have close concentrations in the PHL. The relations between the solid content and lignin or acetic acid content of the PHL are shown in Figure 8. As can be seen, there is a linear relationship between the solid content and lignin or acetic acid concentration of the PHL.

Table IV. Chemical composition and mass balance of industrial PHL samples. (Reproduced with permission from reference (*17*). Copyright 2010 Elsevier)

Sample	*pH*	*Dry Solids*	*Ash (A)*	*Acetic acid (B)*	*UV-lignin (C)*	*Total sugar (D)*	*A+B+C+D*
1	3.68	5.39	1.11	1.31	1.01	1.50	4.93
2	3.63	5.23	1.01	1.39	0.10	1.52	4.92
3	3.67	5.31	1.05	1.52	1.03	1.56	5.16
4	3.63	5.44	1.15	1.21	1.05	1.65	5.06
5	3.70	5.54	1.17	1.33	1.18	1.61	5.29
6	3.73	5.75	1.37	1.44	1.02	1.61	5.43

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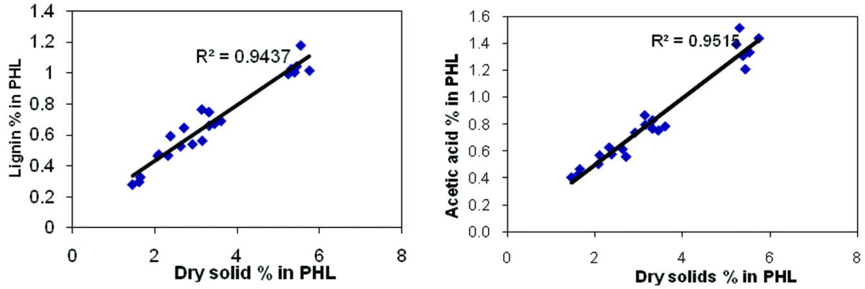

Figure 8. Relationship between the acetic acid or lignin concentration and dry solid content of industrially produced PHL. (Reproduced with permission from reference (17). Copyright 2010 Elsevier).

In the present configuration of the kraft-based dissolving pulp production process, the PHL is burned along with black liquor in the recovery boiler of the mill. Since the heat value of hemicelluloses is almost half of that of lignin, using hemicelluloses as a fuel source is not economical (*1*). The dissolved hemicelluloses in the PHL can be alternatively separated from the liquor, and used in several value-added products. The isolation of hemicelluloses from the liquor and their applications in different products will convert the dissolving pulp production process to an integrated forest biorefinery process, in which the value-added products will increase the revenue of the mill (*40*). To have an economically viable biorefinery process, it is crucial to extract the maximum amount of hemicelluloses from the wood chips prior to the kraft pulping practice, which may in turn improve the quality of the dissolving pulp (*41*). Thus, the following biorefinery processes are proposed for the hydrolysis section of dissolving pulp production processes.

Recovery of Lignocellulosic Materials from PHL

By Flocculation

The relatively low concentration of dissolved materials in the PHL hinders its practical application in various downstream biotechnology processes. Therefore, the dissolved materials of PHL should first be extracted from the liquor. A part of the lignocellulosic materials of the PHL can be separated employing the fundamental concept of colloid science. The flocculation system has been applied in waste water treatments for decades. In this system, the precipitation can be performed by flocculating the lignocellulosic materials via interacting with various cationic chemicals. In one study, poly diallyldimethylammonium chloride (PDADMAC) and chitosan were used for interacting with lignocellulosic materials of the PHL, as shown in Figure 9 (*42*). As can be seen, the maximum removal of materials depended on the polymers applied for the flocculation. The maximum hemicelluloses and furfural removals were obtained via 0.5 mg/g of poly-DADMAC or chitosan addition. However, the maximum removal of lignin was achieved by 1.4 mg/g of PDADMAC or by 1.6 mg/g of chitosan addition to the PHL.

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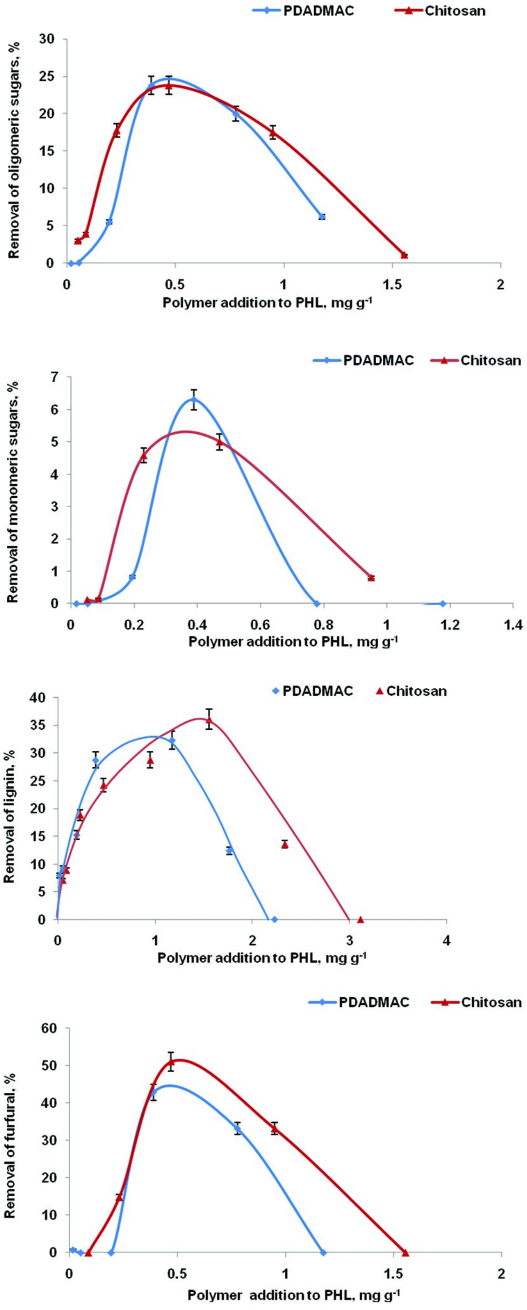

Figure 9. Removal of lignocellulosic materials of industrially produced PHL using various dosages of PDADMAC or chitosan. (room temperature, 10 min., Reproduced with permission from reference 42. Copyright 2011 Elsevier).

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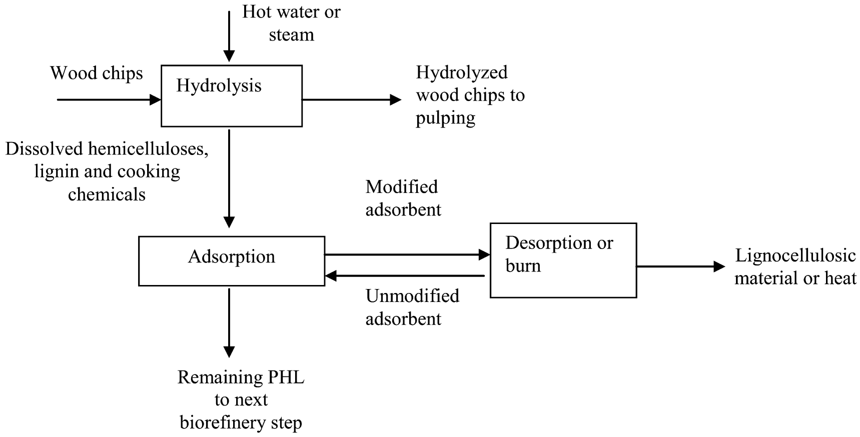
Hot water or steam
Wood chips
Hydrolysis
Hydrolyzed wood chips to pulping
Dissolved hemicelluloses, lignin and cooking chemicals
Modified adsorbent
Adsorption
Desorption or burn
Lignocellulosic material or heat
Unmodified adsorbent
Remaining PHL to next biorefinery step

Figure 10. Proposed process flow diagram of using adsorbent (e.g., activated carbons) for extracting lignocellulosic materials of PHL. (Reproduced with permission from reference (43). Copyright 2011 Elsevier).

The precipitated materials can be used as a solid fuel source of the mill, or may be used as wet end additives in papermaking. The advantage of this system is a fast flocculation process and easily handling of the flocculants by a filter washer. Depending on the amount of extracted lignocellulosic materials required, it may also eliminate the necessity of evaporation. However, the results in Figure 9 show that the total removal of lignocellulosic materials of the PHL was limited. Consequently, the treated PHL can be further processed in downstream stages to produce other value added by-products.

By Adsorption

Alternatively, the extraction of lignocellulosic materials of the PHL can be conducted via employing the fundamental of adsorption/desorption. In this respect, adsorbent materials, e.g., activated carbons or fillers, are known to have a large surface area, which promotes the adsorption. Figure 10 shows the proposed process diagram of adopting an adsorbent for recovering lignocellulosic materials of the PHL.

In this system, the adsorbent is mixed with the PHL to adsorb the lignocellulosic materials under various conditions. Subsequently, the desorption of lignocellulosic materials from the treated adsorbent will take place via applying different chemicals or conditions in another step. The absorbent is usually inert, therefore, can be reused and recycled. This system can practically eliminate the need for using evaporation for concentrating lignocellulosic materials. One main advantage of this system is the flexibility for having certain selectivity towards the adsorption of a desired lignocellulosic material of the PHL. In this regard, activated carbons (ACs) were used for adsorbing lignocellulosic materials of the PHL (*43*). Figure 11 shows the adsorption isotherms of hemicelluloses, lignin and furfural of industrially produced PHL on activated carbons (*43*). Evidently, the final adsorption of hemicelluloses, lignin, and furfural were 400-700 mg/g, 200-300, and 100 mg/g on ACs, respectively, which implies the different selectivity of ACs for various lignocellulosic materials of the PHL. Additionally, the oxidized ACs adsorbed more lignocellulosic materials than the untreated AC did (Figure 11).

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Utilization of Dissolved Lignocellulosic Materials of PHL

Lignin as a Fuel Source

Traditionally, lignin dissolved in black liquor is used as fuel source in the kraft pulp mills (*25*, *32*). As described earlier, the hydrolysis liquor is also burned in the recovery boiler of a dissolving pulp production process. However, the low concentrations of the dissolved lignocellulosic materials of the PHL make this process less economically attractive. Efforts have been made to find alternative ways to utilize these dissolved lignocellulosic materials. Ultrafiltration has been the most received method for separating lignin from the black liquor of kraft pulping (*44*, *45*). It is a pressure-driven technique that has been used in industry for various purposes (*45*). Principally, it can be used for separating solute that has at least one or two order of magnitude larger size than a solvent does. Similarly, ultrafiltration can be adopted for separating the dissolved lignin of the PHL. The typical problems of ultrafiltration are flux, membrane fouling, and the formation of gel layers. The advantage of ultrafiltration is that the pH or temperature adjustments are not required and the molecular weight of the products can be controlled. However, since the molecular weight of lignin is similar to that of other dissolved organics of the PHL, the selectivity of ultrafiltration for removing lignin is not very high. It is also a slow and costly process.

Alternatively, calcium oxide, which is available in the recovery unit of kraft process, can be added to the PHL to neutralize the PHL. During this process, lignin may also adsorb on the calcium oxide/hydroxide particles. The mixtures can be burned in the lime kiln of the recovery section of the mill. This process is shown in Figure 12.

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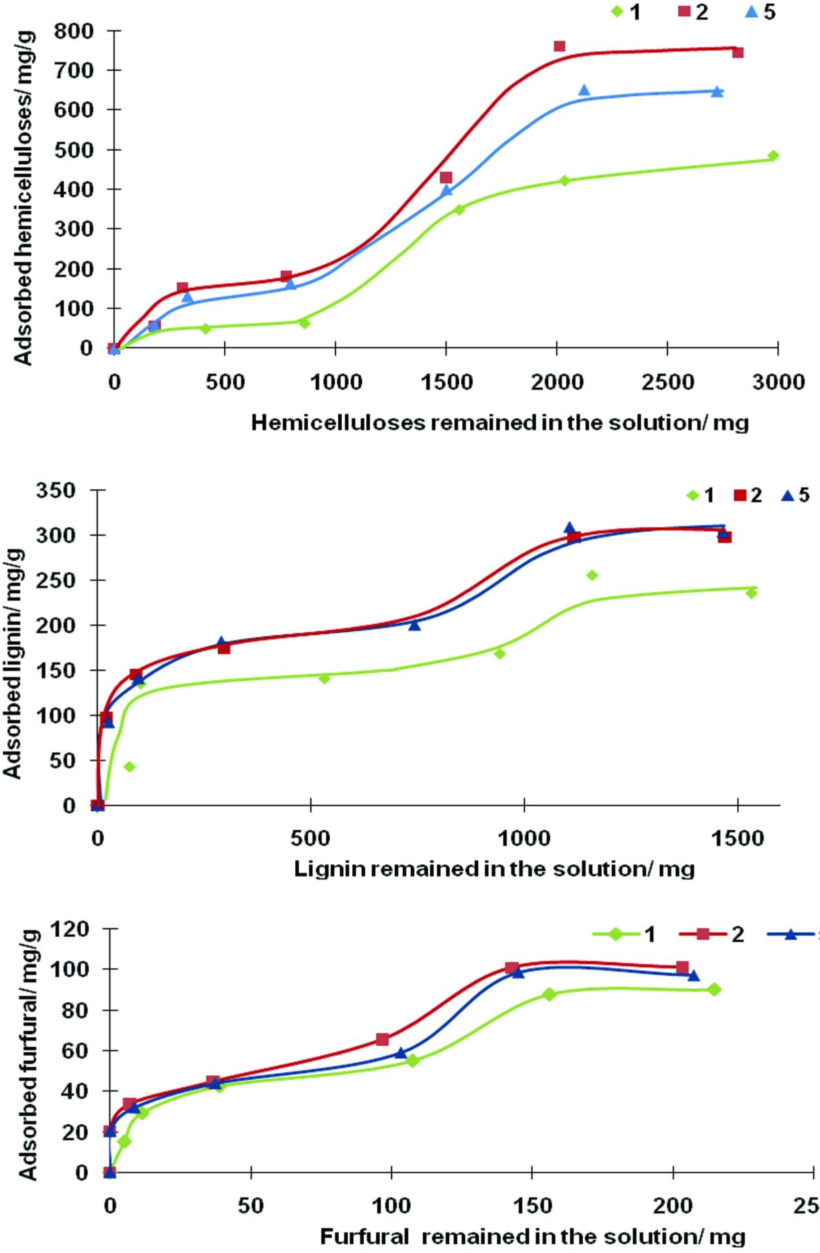

Figure 11. Adsorption of various lignocellulosic materials of the PHL on different activated carbons conducted at room temperature for 24 h at 150 rpm. 1) Untreated AC, 2) H_2O_2-oxidized AC, 5) H_2SO_4-oxidized AC. (Reproduced with permission from reference (43). Copyright 2011 Elsevier).

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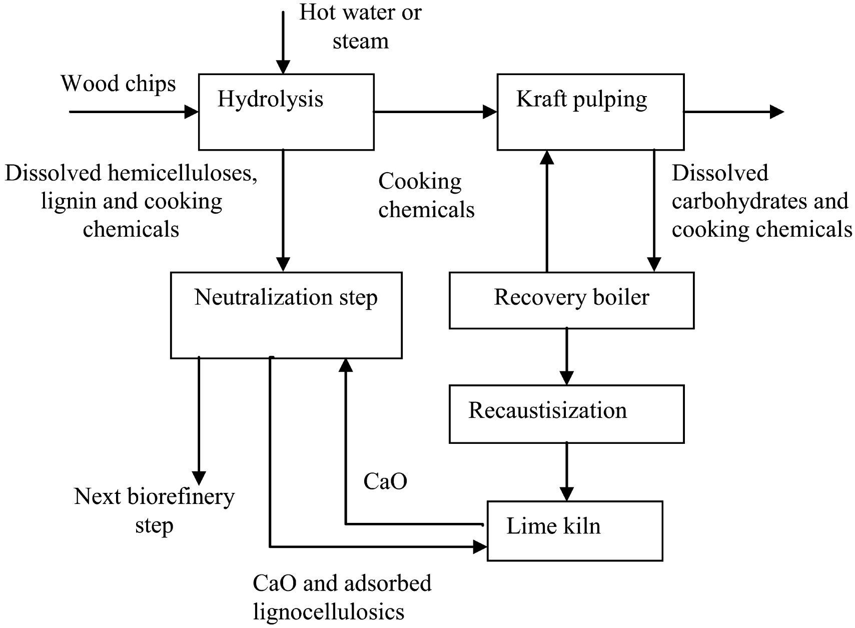
Hot water or steam
Wood chips
Hydrolysis
Kraft pulping
Dissolved hemicelluloses, lignin and cooking chemicals
Cooking chemicals
Dissolved carbohydrates and cooking chemicals
Neutralization step
Recovery boiler
Recaustisization
CaO
Next biorefinery step
Lime kiln
CaO and adsorbed lignocellulosics

Figure 12. Proposed process flow diagram of lignin isolation using lime in neutralization step of PHL.

Lignin precipitation may be improved by using other chemicals, such as surfactants. The interaction of surfactants with hydrophobic materials has been well documented in the literature (*46*, *47*). The hydrophobicity of lignin helps its flocculation with surfactants. The flocculants can then be separated via centrifuging or filtering, and used as fuel source. Table V shows the concentration of lignin and hemicelluloses via adjusting pH of the PHL using $Ca(OH)_2$ (*48*). These results showed that the concentration of lignin and hemicelluloses were reduced by 43% and 26.8%, respectively, by adjusting the pH to 12 (overliming).

Lignin Recovery for Producing Various By-Products

As described in Chapter 10, lignin has a variety of industrial applications. To facilitate the application of lignin, it should be first extracted from the PHL. It seems that the most adoptable method for separating lignin is to acidify the solution for promoting the precipitation of acid insoluble lignin. The separation of precipitated lignin will then be achieved in a filter washer.

Table VI lists the mass balance of the acid treatment of the PHL obtained from a dissolving pulp production process via applying sulfuric acid for adjusting the pH to 2 (*49*). Evidently, about 50% of lignin was separated via pH adjustment, but carbohydrate removal was 16%. Further acid washing of the precipitates may help purify precipitated lignin. However, it has been well documented that the lignin removal/precipitation via the acidification was significantly affected by the structure/functional groups and molecular weight of the dissolved lignin in the

PHL (*50*, *51*). Therefore, the acidification process may not be a very efficient method for extracting lignin of different PHLs.

Table V. Concentrations of lignin and hemicelluloses via adjusting pH of PHL using $Ca(OH)_2$. (Reproduced with permission from reference (*48*). Copyright 2011 Wiley)

pH	*Dodeyltrimethylammonium chloride/PHL (%)*	*Lignin, g/l*	*Sugars, g/l*
2	0	10.2	26.3
2	0.3	9.7	25.5
3.5	0	11.3	30.0
3.5	0.3	9.4	26.4
7	0	10.0	25.4
7	0.3	9.1	24.1
9	0	8.7	23.4
9	0.3	7.8	22.1
12	0	7.3	23.1
12	0.3	6.4	21.9

Table VI. Main component of PHL before and after acidification conducted at a room temperature. (Reproduced with permission from reference (*49*). Copyright 2011 Elsevier)

	Original solution	*Precipitate after acidification*	*Supernatants after acidification*
Solid content (% wt.)	0.83	0.18	0.62
Ash	0.11	0.01	0.10
Lignin	0.34	0.16	0.17
Carbohydrates	0.38[a]	0.05[b]	0.23[b]

[a] sugars+rest of dissolved organic materials. [b] sugars.

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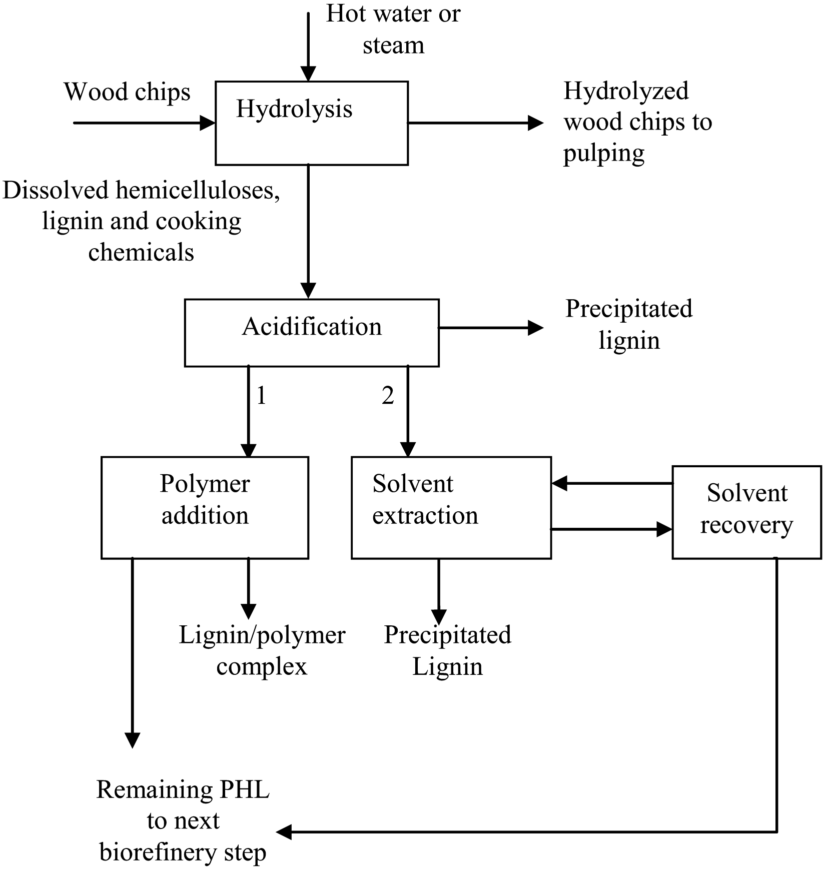
Hot water or steam
Wood chips
Hydrolysis
Hydrolyzed wood chips to pulping
Dissolved hemicelluloses, lignin and cooking chemicals
Acidification
Precipitated lignin
1
2
Polymer addition
Solvent extraction
Solvent recovery
Lignin/polymer complex
Precipitated Lignin
Remaining PHL to next biorefinery step

Figure 13. Proposed process flow diagram of lignin isolation from PHL. (Reproduced with permission from reference (49). Copyright 2011 Elsevier).

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The remaining lignin present in the acidified PHL can be further removed by interacting with some chemicals. Figure 13 shows a process flow diagram for separating lignin from the PHL. In this Figure, stream 1 is for the possibility of using polymers (e.g., Poly ethylene oxide (PEO), Poly aluminum chloride (PAC)) to form complexes with lignin, which facilities the lignin flocculation and precipitation. The precipitates of PEO/PAC/lignin may be used as wet end additives (retention aids) for papermaking. Stream 2 depicts the possibility for precipitating lignin via a solvent extraction (e.g., ethyl acetate (EC) or ether). As the precipitated lignin via acidification and solvent extraction steps possess a very high purity, it can be further utilized in different processes for the production of various value-added products. The solvent should then be recovered from the PHL, which may be used in downstream biorefinery processes.

To induce the chemical interaction, ethyl acetate (EC) or additives (PEO, PAC) were added to the acidified PHL samples in one study (*49*). The further removal of lignin (%) after each treatment is listed in Table VII. It is evident that the addition of ethyl acetate (EC) to the acidified PHL significantly improved the lignin removal (64%), and a higher ratio of ethyl-acetate/PHL (v/v) induced a more lignin precipitation.

Acetic Acid Recovery

As mentioned earlier (Table IV), acetic acid contributes to almost 25% of the total dissolved materials of the PHL. In the literature, the solvent extraction technique via applying ethyl acetate or methyl isobutyl ketone was used for extracting acetic acid from solvent mixtures, as shown in Figure 14 (*13*, *52*, *53*). In this system, the PHL is sent to a solvent extraction unit, in which acetic acid is transferred to the solvent. Then, solvent is separated from the PHL mixture and sent to the solvent recovery section. The recovered solvent is recycled into the system.

However, the recovery of solvent necessitates the existence of complex processes in the pulp mill. Additionally, the concentration of acetic acid is rather low in the PHL (about 1%) (See Figure 8 and Table IV), which hinders the feasibility and adoptability of the solvent extraction system in industrial scales. However, the incentive for its separation from the PHL is high, as it is a value added chemical.

Table VII. Precipitation of lignin (%) from acidified PHL using various chemicals or solvents conducted at a room temperature. (Reproduced with permission from reference (*49*). Copyright 2011 Elsevier)

Sample	*Type*	*Treatment condition*	*Lignin removal, %*
3	Ethyl-acetate (EC)	EC/PHL=1.5	54.7
4	EC	EC/PHL=4	64.3
5	PEO	D[a]=10 ppm	0
6	PEO	D=25 ppm	0
7	PEO	D=50 ppm	0
8	PEO	D=100 ppm	4.4
9	PAC	D=100 ppm	0
10	PEO/PAC	D=10ppm/100 ppm	2
11	PEO/PAC	D= 25 ppm/100 ppm	3
12	PEO/PAC	D=50 ppm/100 ppm	10.6
13	PEO/PAC	D=100/100	13.2

[a] Dosage.

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Furfural Recovery

As mentioned earlier, furfural is also a value added product, which can be extracted from hydrolysis liquor. To obtain furfural from the PHL, first hemicelluloses should be converted to furfural, which can be conducted at an elevated temperature under acidic conditions (Figure 15). Furfural can then be obtained by using a distillation system. The advantage of this method is the high purity of furfural collected. However, the complexity (specially the high cost) of the process may be a barrier for its adaptation in industrial scales.

Hemicelluloses Recovery

Hemicelluloses recovery is the first step of producing several value added products from the PHL, which makes its separation and purification of great importance. Hemicelluloses can be separated from the PHL via two potential pathways, as shown in Figure 16. In this system, a solvent, e.g., ethanol or acetone, promotes the precipitation of hemicelluloses from the PHL. In the literature, it was reported that adding ethanol to dissolved hemicelluloses solutions effectively facilitated the precipitation and separation of hemicelluloses (*12*, *54*). As can be seen in Stream 1 of Figure 16, the PHL can be acidified to remove lignin, which is followed by a solvent extraction for separating hemicelluloses.

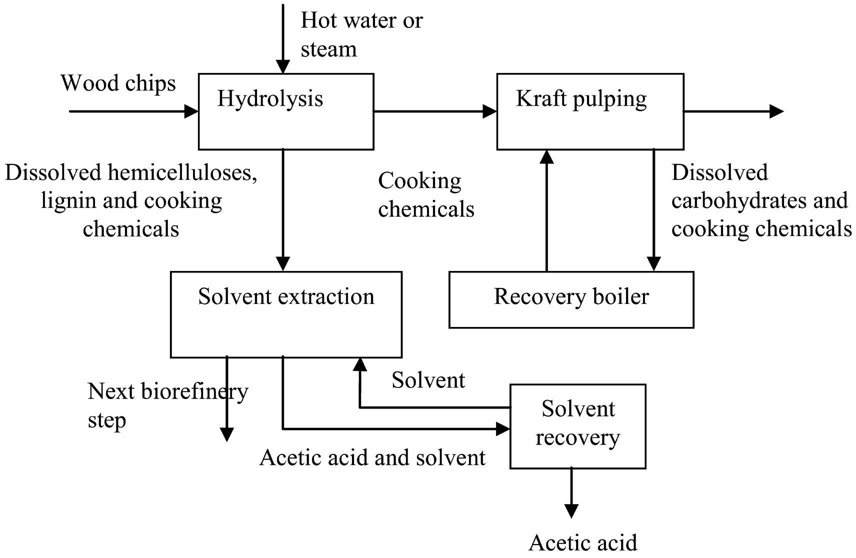
Hot water or steam
Wood chips
Hydrolysis
Kraft pulping
Dissolved hemicelluloses, lignin and cooking chemicals
Cooking chemicals
Dissolved carbohydrates and cooking chemicals
Solvent extraction
Recovery boiler
Solvent
Next biorefinery step
Solvent recovery
Acetic acid and solvent
Acetic acid

Figure 14. Proposed process flow diagram of acetic acid recovery from PHL. (Reproduced with permission from reference (13). Copyright 2008 de Gruyter).

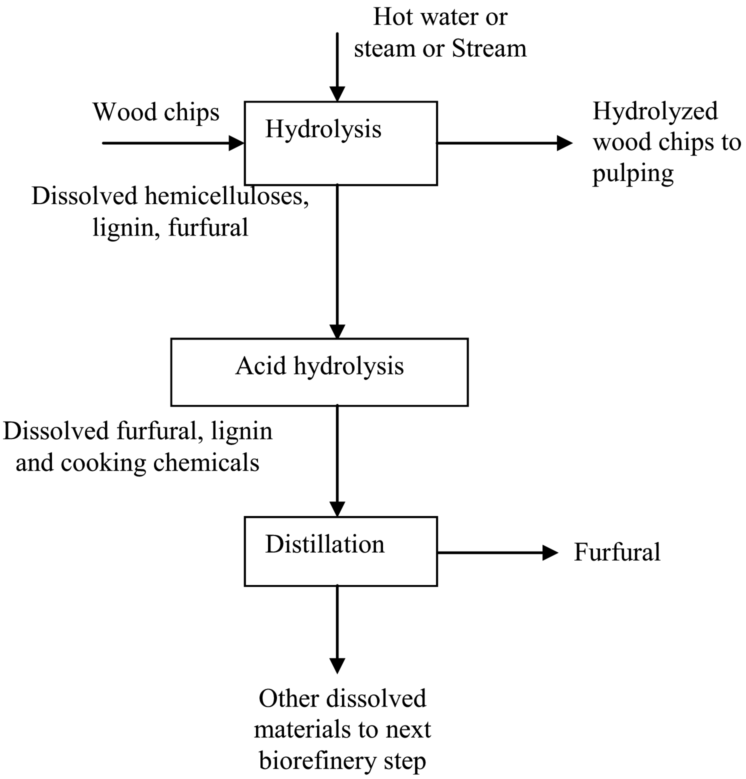
Hot water or steam or Stream
Wood chips
Hydrolysis
Hydrolyzed wood chips to pulping
Dissolved hemicelluloses, lignin, furfural
Acid hydrolysis
Dissolved furfural, lignin and cooking chemicals
Distillation
Furfural
Other dissolved materials to next biorefinery step

Figure 15. Proposed process flow diagram for extracting furfural from PHL.

The proposed method was discussed in the literature (*49*). Table VIII shows the characteristics of hemicelluloses isolated from the acidified PHL. It can be found that the hemicelluloses and lignin contents of the isolated hemicelluloses samples were 86.25% and 0.44%, respectively, which confirmed the high selectivity of using ethanol for isolating hemicelluloses. Additionally, xylose/xylan was the predominant sugar component (46.7%) of the hemicelluloses isolated. However, the proposed method requires a distillation system to recover the solvent, which may hinder its further application in industrial scales. The feasibility of applying the above method depends on the revenue of the by-products obtained.

Alternatively, Stream 2 in Figure 16 shows that the solvent extraction can be directly applied on the PHL. In this system, lignin is not separated prior to removing hemicelluloses. Thus, the dissolved high molecular weight lignin may also be separated during the solvent extraction process. The extracted hemicelluloses may require additional bleaching stages for removing lignin. The bleaching is conducted using the existing facilities and bleaching chemicals of the kraft-dissolving pulping process. Table IX lists the results of bleaching conducted on the precipitates obtained from the above-mentioned system via Stream 2 of Figure 16 (*55*). As can be seen, the yield of bleaching and the brightness obtained are rather low, which is due to the high lignin content of precipitates via Stream 2.

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The hemicelluloses content is about 81% of the total solid, while the lignin content is about 5%. Furthermore, the hemicelluloses content along with lignin content were reduced in each step of subsequent bleaching stages. The final lignin content was 2.5%, which caused the rather low brightness of precipitates at the final stage. This analysis implies that one step ethanol extraction process for separating hemicelluloses results in a high lignin content in the hemicelluloses obtained. If the extracted hemicelluloses are to be applied as polymers/additives, the rather low brightness of the extracted hemicelluloses may limit their applications. For example, they can only be employed as strength additives for low-brightness paperboard, e.g., recycled fibers or NSSC- based paperboard products. However, if they are to be depolymerized to monomers and the monomers are the raw materials for downstream products, extracted hemicelluloses may still serve for such purposes.

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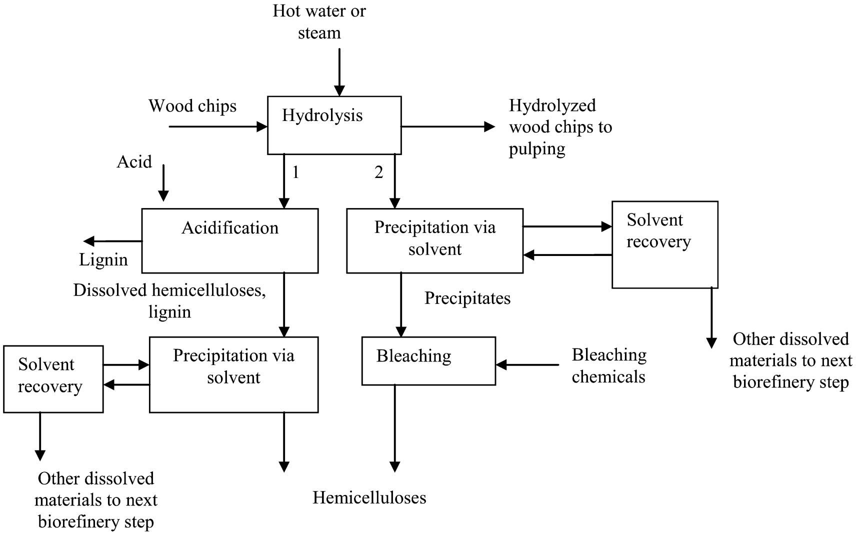
Hot water or steam
Wood chips
Hydrolysis
Hydrolyzed wood chips to pulping
Acid
1
2
Acidification
Lignin
Precipitation via solvent
Solvent recovery
Dissolved hemicelluloses, lignin
Precipitates
Solvent recovery
Precipitation via solvent
Bleaching
Bleaching chemicals
Other dissolved materials to next biorefinery step
Other dissolved materials to next biorefinery step
Hemicelluloses

Figure 16. Proposed process flow diagram of hemicelluloses extraction from PHL. (Reproduced with permission from references (49) and (55). Copyright 2011 Elsevier and Wiley, respectively).

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Table VIII. Chemical composition of the hemicelluloses obtained via ethanol extraction of acidified PHL. (Reproduced with permission from reference (*49*). Copyright 2011 Elsevier)

Ash Content (%)		8.00
Lignin Content (%)		0.44
Sugar Content (%)	Arabinose	1.7 (2.0)[1]
	Galactose	6.0 (7.0)
	Glucose	21.1 (24.5)
	Xylose	46.7 (54.1)
	Mannose	10.7 (12.4)
	Total	86.2

[1] The values in parenthesis represent the sugar percentage in the total sugar determined.

Ethanol Production

When hardwoods are used for producing dissolving pulp in a kraft-based process, the dissolved hemicelluloses are mainly pentose in the PHL. The ethanol production based on lignocellulosic materials has been comprehensively documented in the literature (*40*, *56–58*). Generally, the productivity of ethanol production via fermentation is high for hexose sugars, but is rather low for pentose sugars. However, the relatively high concentration of sugars (even pentoses) in the PHL indicates that ethanol can be a potential by-product. In fact, it was claimed as the most promising value-added product induced from the PHL of kraft-based dissolving pulp production process (*40*, *56*). Research on the production of ethanol from the PHL is at research and development stage. To produce ethanol from the PHL, several steps may be required after extracting hemicelluloses. Figure 17 shows the block diagram of ethanol production from the PHL.

As can be seen, the lignocellulosic materials dissolved in the PHL should be first converted to monomers (*35*). The treatment of the PHL by using sulfuric acid at a low pH (<2) for 1 h could convert oligomeric sugars to monomeric sugars (*27*, *59*, *60*). A purification or detoxification stage is then required as some of the products of hydrolysis, e.g., acetic acid, formic acid, furfural and its derivatives, phenolic/aromatic compounds, inhibit the activity of enzymatic activities/fermentation for the ethanol production (*30*, *61*, *62*). It has been shown that an overliming treatment can lead to the soluble lignin destruction and removal of furfural and HMF, which reduced the toxicity and improved the fermentability of the PHL (*59*, *63*, *64*). Neutralization with lime has the advantage of forming insoluble salt with sulfuric acid (gypsum) and thus reducing the concentration of soluble salt during fermentation (*59*, *63*, *64*). The PHL can also be neutralized by using sodium or calcium hydroxide to produce ethanol from the hydrolysis liquor (*65*).

In Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass; Zhu, J., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

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Table IX. The properties of hemicelluloses obtained from industrially produced PHL at different stage of bleaching sequence (D_0EpD_1). (Reproduced with permission from reference (*55*). Copyright 2011 Wiley)

Conditions		*Hemicelluloses from PHL*	*D_0*	*D_1*
ClO_2 dosage, %(wt.)		-	2.5	0.6
Temperature, ºC		-	60	70
Time, h		-	2	3
Initial pH		-	2-3	7
Properties				
Brightness, (ISO%)		17.2	36.6	59.5
Yield, %		100	53.6	74.3
Lignin Content (%)		5.67	3.76	2.50
Hemicelluloses Content (%)	Arabinose	1.93	0.90	0.56
	Galactose	3.91	2.77	3.01
	Glucose	11.66	11.88	12.07
	Xylose	54.89	49.01	45.43
	Mannose	9.04	11.81	12.92
	Total	81.43	76.37	73.99

The results presented in Table V confirmed the detoxification of overliming of industrially produced PHL. However, it significantly decreased the initial sugars concentration, which may hinder the subsequent ethanol production. The detoxification can also be conducted using ion exchange resin (*66*). Subsequently, the mono sugars should be converted to ethanol via fermentation or enzymatic treatments. In the literature, a variety of microorganism, e.g., *C.shehatae, P.tannophilus*, were reported to ferment carbohydrate to ethanol under oxygen free conditions (*67*). The advantage of this system is that ethanol can be the by-product of the process, and also used in the solvent extraction for precipitating hemicelluloses (dash line in Figure 17). Thus, this is an integrated system in that it does not necessarily require outsources for ethanol supply, which will help the revenue of the mill.

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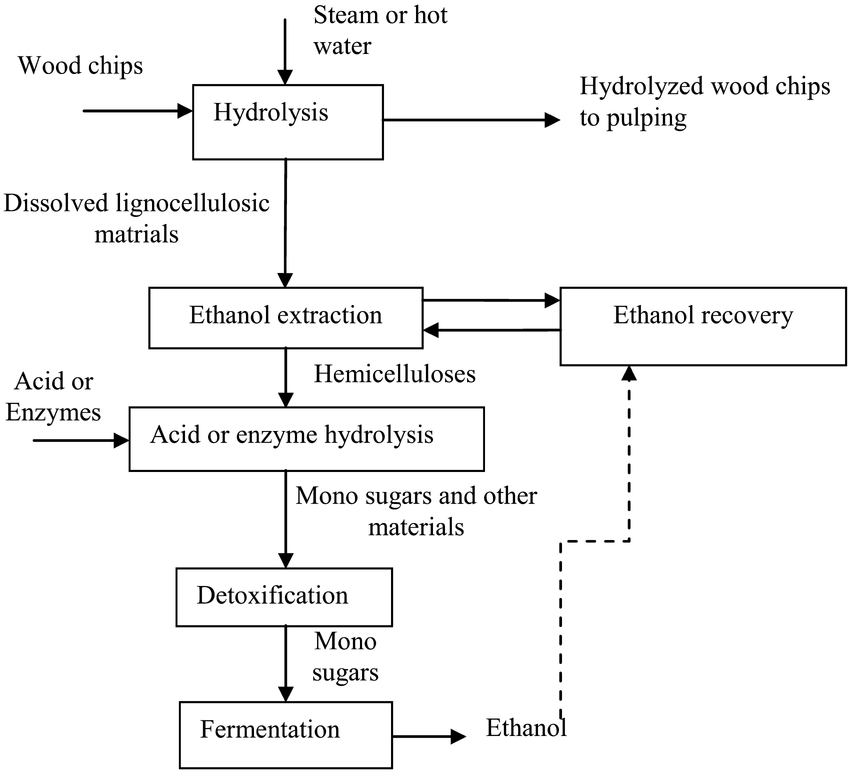
Steam or hot water
Wood chips
Hydrolysis
Hydrolyzed wood chips to pulping
Dissolved lignocellulosic matrials
Ethanol extraction
Ethanol recovery
Hemicelluloses
Acid or Enzymes
Acid or enzyme hydrolysis
Mono sugars and other materials
Detoxification
Mono sugars
Fermentation
Ethanol

Figure 17. Proposed process flow diagram of ethanol production from PHL. (Reproduced with permission from reference (35). Copyright 2010 Elsevier).

Lactic Acid, Succinate, and Protein Productions

The production of mono sugars (pentoses) promotes the production of various value added products from the PHL. It was also well documented in the literature that the impurities, e.g., lignin, furfural, strongly impaired the efficiency of the production of various value added products from the PHL (*31*, *35*, *61*). Therefore, purification steps should be conducted on the hemicelluloses extracted from the PHL prior to producing lactic acid, succinate, and proteins. It was reported that the mono sugars can be converted to lactic acid using *Bacillus coagulans* MXL-9 via pentose-phosphate pathway (*31*). Figure 18 shows the production rate of lactic acid and the consumption of various sugars versus time. As can be seen, by increasing the time of treatment with *Bacillus coagulans* MXL-9, the concentration of lactic acid increased, while the sugar concentrations decreased.

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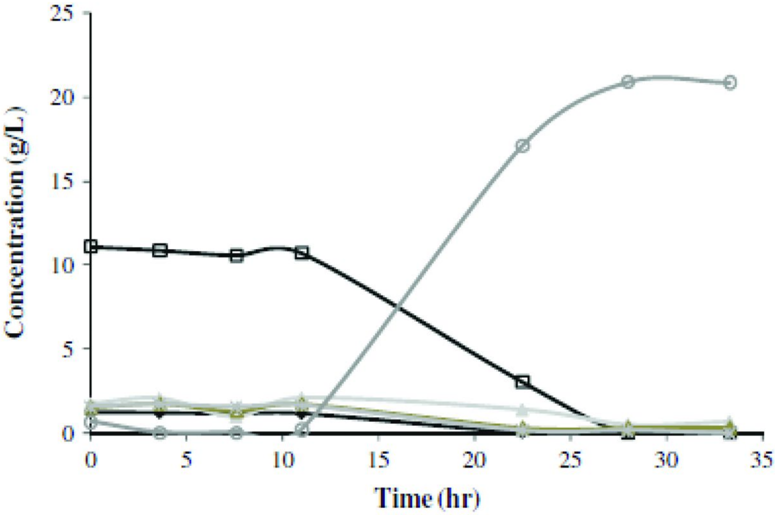

Figure 18. Fermentation of PHL from hot water extraction of southern hardwood by using Bacillus coagulans MXL-9 to produce lactic acid (circle), and the concentration decreases of glucose (filled diamond), mannose (star), xylose (square box), arabinose (triangle), galactose (filled triangle). (Reproduced with permission from reference (31). Copyright 2010 Springer).

If *E.coli* is used in the fermentation of mono sugars, succinate can be produced. In this system, *E.coli* consumes both acetate and xylose as substrate for cell growth in aerobic growth phase (*35*). However, the remaining xylose is converted to succinate under anaerobic conditions, as shown in Figure 19.

In another study, the mono sugars obtained from the hydrolysis of the hemicelluloses of the PHL were consumed for folder yeast (protein) culture using *Canadida scottii* yeast (*68*). The impurities affected the process, which necessitated pre-purification steps in the process (*68*).

Xylitol Production

Xylitol has several medical and pharmaceutical applications, e.g., oral hygiene and dietetic products and cosmetics (*69*, *70*). There are two potential pathways to produce xylitol from hemicelluloses in the PHL, as shown in Figure 20. In Stream 1, xylose is first produced and purified according to the above-mentioned procedure from the PHL. The produced xylose can be converted to xylitol via hydrogenation in the presence of Raney nickel catalyst (*69*)(*70*). The produced xylitol can then be crystallized from the hydrogenated solution. The uncrystilized xylitol fraction is separated using a liquid chromatography in a column containing cationic exchange resins (calcium or strontium). The obtained filtrate of chromatographic column can possess various polyols including mannitol, arabitol, galactitol and sorbitol (*69*, *70*). The fraction having a very high xylitol concentration is crystallized and the xylitol is collected (*69*) (Stream 1 in Figure 20). Alternatively, the obtained xylose can be converted to xylitol via using *Candida tropicalis* strain (AS2.1776) (Stream 2 in Figure 20) (*71*).

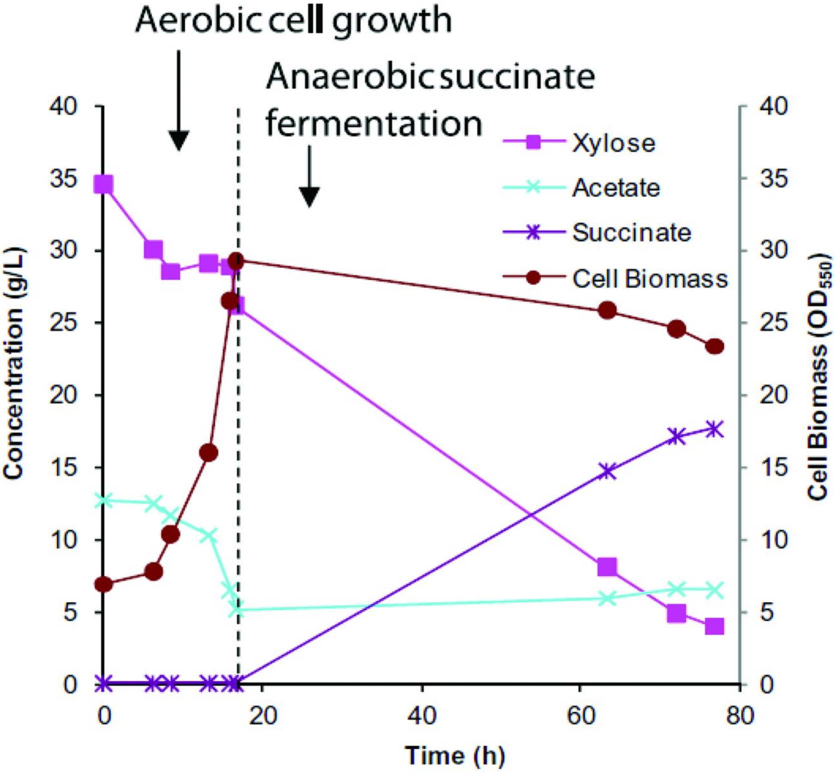

Figure 19. Fermentation of PHL from hot water extraction of birch to produce succinate by metabolically engineered E.coli AFP 184. (Reproduced with permission from reference (35). Copyright 2010 Elsevier).

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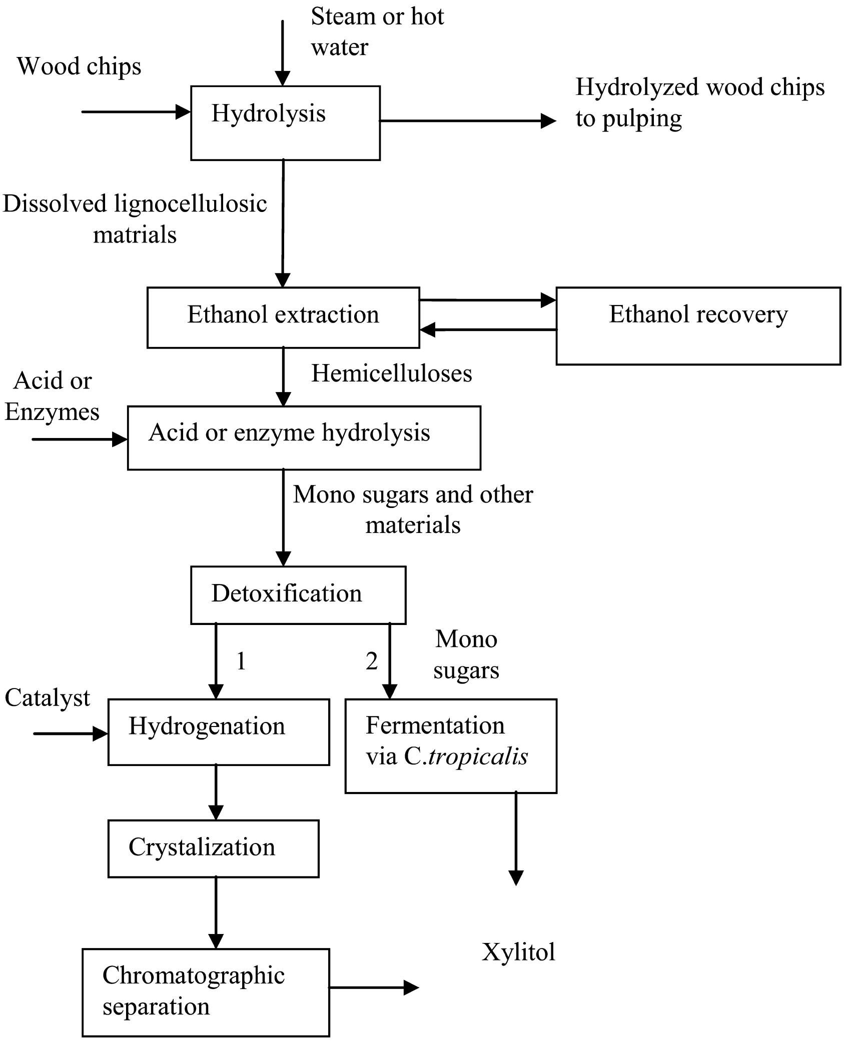

Steam or hot water
Wood chips
Hydrolysis
Hydrolyzed wood chips to pulping
Dissolved lignocellulosic matrials
Ethanol extraction
Ethanol recovery
Hemicelluloses
Acid or Enzymes
Acid or enzyme hydrolysis
Mono sugars and other materials
Detoxification
1
2
Mono sugars
Catalyst
Hydrogenation
Fermentation via C.*tropicalis*
Crystalization
Xylitol
Chromatographic separation

Figure 20. Proposed process flow diagram of producing xylitol from hemicelluloses in PHL.

References

1. Van Heiningen, A. *Pulp Pap. Can.* **2006**, *107* (6), 38–43.
2. Chambost, V.; Eamer, B.; Stuart, P. *Pulp Pap. Can.* **2007**, *108* (6), 30–35.
3. Amidon, T. E. *Pulp Pap. Can.* **2006**, *107* (6), 47–50.
4. Stuart, P. *Pulp Pap. Can.* **2006**, *107* (6), 13–16.
5. Wising, U.; Stuart, P. *Pulp Pap. Can.* **2006**, *107* (6), 25–30.
6. Ragauskas, A. J.; Nagy, M.; Kim, D. H.; Eckert, C. A.; Hallett, J. P.; Liotta, C. L. *Ind. Biotechnol.* **2006**, *2* (1), 55–65.

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7. Cruz, J. M.; Dominguez, J. M.; Dominguez, H.; Parajo, J. C. *Food Chem.* **1999**, *67*, 147–153.
8. Amidon, T. E.; Wood, C. D.; Shupe, A. M.; Wang, Y.; Graves, M.; Liu, S. *J. Biobased Mater. Bioenergy* **2008**, *2* (2), 100–120.
9. Carvalheiro, F.; Durate, L. C.; Girio, F. M. *J. Sci. Ind. Res.* **2008**, *67*, 849–864.
10. Alvarado-Morales, M.; Terra, J.; Gernaey, K. V.; Woodley, J. M.; Gani, R. *Chem. Eng. Res. Des.* **2009**, *87*, 1171–1183.
11. Huang, H. J.; Ramaswamy, S.; Al-Dajani, W. W.; Tschirner, U. *Bioresour. Technol.* **2010**, *101*, 624–631.
12. Ren, J. L.; Peng, F.; Sun, R. C. *J. Biobased Mater. Bioenergy* **2009**, *3*, 62–68.
13. Leschinsky, M.; Zuckerstatter, G.; Weber, H. K.; Patt, R.; Sixta, H. *Holzforschung* **2008**, *62*, 645–652.
14. Mao, H.; Genco, J. M.; Yoon, S. H.; van Heiningen, A.; Pendse, H. *J. Biobased Mater. Bioenergy* **2008**, *2* (2), 177–185.
15. Tunc, S. M.; van Heiningen, A. *Ind. Eng. Chem. Res.* **2008**, *47* (18), 7031–7037.
16. Wang, S.; Wang, K.; Liu, Y.; Luo, Z.; Cen, K.; Fransson, T. *Biotechnol. Adv.* **2009**, *27*, 562–567.
17. Saeed, A., Jahan, M. S., Li, H., Liu, Z., Ni, Y., van Heiningen, A. P. R. *Biomass Bioenergy* 2010, doi:10.1016/j.biombioe.2010.08.039.
18. Jonsson, A. S.; Wallberg, O. *Desalination* **2009**, *237*, 254–267.
19. Amidon, T. E.; Liu, S. *Biotechnol. Adv.* **2009**, *27*, 542–550.
20. Vanderlaan, M. N.; Thring, R. W. *Biomass Bioenergy* **1998**, *14* (5/6), 525–531.
21. Bonini, C.; Dauria, M.; Ferri, R.; Pucciariello, R.; Sabia, A. R. *J. Appl. Polym. Sci.* **2003**, *90*, 1163–1171.
22. Kumar, M. N. S.; Mohanty, A. K.; Erickson, L.; Misra, M. *J. Biobased Mater. Bioenergy* **2009**, *3*, 1–24.
23. Rozman, H. D.; Tan, K. W.; Kumar, R. N.; Abubakar, A. *J. Appl. Polym. Sci.* **2001**, *81*, 1333–1340.
24. Janzon, R.; Puls, J.; Saake, B. *Holzforschung* **2006**, *60*, 347–354.
25. Sixta, H. In *Hand Book of Pulp*, 1st ed.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2006; Volume 1, 325−365.
26. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y. Y.; Holtzapple, M.; Ladisch, M. *Bioresour. Technol.* **2005**, *96*, 673–686.
27. Liu, S.; Mishra, G.; Amidon, T. E.; Gratien, K. *J. Biobased Mater. Bioenergy* **2009**, *3*, 363–372.
28. Tunc, M. S.; Lawoko, M.; van Heiningen, A. *Bioresources* **2010**, *5* (1), 356–371.
29. Ebringerova, A.; Hromadkova, Z. *Biotechnol. Genet. Eng. Rev.* **1999**, *16*, 325–346.
30. Sun, R. C. *Bioresources* **2008**, *4* (2), 452–455.
31. Walton, S. L.; Bischoff, K. M.; van Heiningen, A. R. P.; van Walsum, G. P. *Ind. Microbiol. Biotechnol.* **2010**, *37* (8), 823–830.

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32. Gullichsen, J., Fogelholm, C. J. In *Papermaking Science and Technology, Chemical Pulping*, Book 6A; Gullichsen, J., Paulapuro, H., Eds.; Fapet Oy: Jyvaskyla, Finland, 1999; Chapters 3−6.
33. Leschinsky, M.; Zuckerstatter, G.; Weber, H. K.; Patt, R.; Sixta, H. *Holzforschung* **2008**, *62*, 653–658.
34. Li, H.; Saeed, A.; Ni, Y.; van Heiningen, A. R. P. *J. Wood Chem. Technol.* **2010**, *30* (1), 48–60.
35. Helmerius, J.; von Walter, J. V.; Rova, U.; Berglund, K. A.; Hodge, D. B. *Bioresour. Technol.* **2010**, *101*, 5996–6005.
36. Girio, F. M.; Fonseca, C.; Carvalheiro, F.; Duarte, L. C.; Marques, S.; Bogel-Lukasik, R. *Bioresour. Technol.* **2010**, *101*, 4775–4800.
37. Leschinsky, M.; Sixta, H.; Patt, R. *Bioresources* **2009**, *4* (2), 687–703.
38. Chen, X.; Lawoko, M.; van Heiningen, A. *Bioresour. Technol.* **2010**, *101*, 7812–7819.
39. Carrasco, F.; Roy, C. *Wood Sci. Technol.* **1992**, *26*, 189–208.
40. Wyman, C. E. *Annu. Rev. Energy Environ.* **1999**, *24*, 189–226.
41. Duff, S.; Murray, W. *Bioresour. Technol.* **1996**, *55*, 1–33.
42. Saeed, A., Fatehi, P., Ni, Y. J. *Biomass Bioenergy* **2011**, submitted.
43. Liu, X., Fatehi, P., Ni, Y. *Chem. Eng. J.* **2011**, submitted.
44. Kirkman, A. G.; Gratzl, J. S.; Edwards, L. L. *Tappi* **1986**, *69* (5), 110–114.
45. Wallberg, O.; Jonsson, A. S.; Wimmerstedt, R. *Desalination* **2003**, *156*, 145–153.
46. Eriksson, T.; Borjesson, J.; Tjerneld, F. *Enzyme Microb. Technol.* **2002**, *31* (3), 353–364.
47. Somasundaran, P.; Zhang, L. *J. Pet. Sci. Eng.* **2006**, *52* (1−4), 198–212.
48. Fatehi, P., Shi, H., Xiao, H., Ni, Y. *Biomass Bioenergy* **2011**, submitted.
49. Liu, Z.; Fatehi, P.; Jahan, M. S.; Ni, Y. *Bioresour. Technol.* **2011**, *102*, 1264–1269.
50. Toledano, A.; Serrano, L.; Garcia, A.; Mondragon, I.; Labidi, J. *Chem. Eng. J.* **2010**, *157*, 93–99.
51. García, A.; Toledano, A.; Serrano, L.; Egüés, I.; González, M.; Marín, F.; Labidi, J. *Sep. Pur. Technol.* **2009**, *68*, 193–198.
52. Lee, Y. M.; Kang, J. S.; Nam, S. Y.; Choi, C. H. *Sep. Sci. Technol.* **2001**, *36* (3), 457–471.
53. Mao, H.; Genco, J. M.; van Heiningen, A.; Pendse, H. *Bioresources* **2010**, *5* (2), 525–544.
54. Tunc, M. S.; van Heiningen, A. R. P. *Holzforschung* **2008**, *62* (5), 539–545.
55. Liu, Z., Fatehi, P., Ni, Y. *Chem. Eng. J.* **2011**, submitted
56. Kaylen, M.; van Dyne, D. L.; Choi, Y. S.; Blasé, M. *Bioresour. Technol.* **2000**, *72*, 19–32.
57. Huang, H. J.; Ramaswamy, S.; Tschirner, U. W.; Ramarao, B. V. *Sep. Pur. Technol.* **2008**, *62*, 1–21.
58. Sammons, N. E. J.; Yuan, W.; Eden, M. R.; Aksoy, B.; Cullinan, H. T. *Chem. Eng. Res. Des.* **2008**, *86*, 800–808.
59. Larsson, S.; Palmqvist, E.; Hahn-Hagerdal, B.; Tengborg, C.; Stenberg, K.; Zacchi, G.; Nilvebrant, N. O. *Enzyme Microb. Technol.* **1999**, *24*, 151–159.
60. Kleinert, M.; Barth, T. *Energy Fuels* **2008**, *22*, 1371–1379.

61. Hahn-Hagerdal, B.; Karhumaa, K.; Fonseca, C.; Spencer-Martins, I.; Gorwa-Grauslund, M. F. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 937–953.
62. Miller, E. N.; Jarboe, L. R.; Turner, P. C.; Pharkya, P.; Yomano, L. P.; York, S. W.; Nunn, D.; Shanmugam, K. T.; Ingram, L. O. *Appl. Environ. Microbiol.* **2009**, *75* (19), 6132–6141.
63. Martinez, A.; Rodriguez, M. E.; Wells, M. L.; York, S. W.; Perston, J. F.; Ingram, L. O. *Biotechnol. Prog.* **2001**, *17*, 287–293.
64. Martinez, A.; Rodriguez, M. E.; York, S. W.; Perston, J. F.; Ingram, L. O. *Biotechnol. Bioenergy* **2000**, *69*, 526–536.
65. Mendes, C. V. T.; Carvalho, M. G. V. S.; Baptista, C. M. S. G.; Rocha, J. M. S.; Soares, B. I. G.; Sousa, G. D. A. *Food Bioprod. Process.* **2009**, *87*, 197–207.
66. Zhuang, J.; Lin, L.; Liu, J.; Luo, Z.; Pang, C.; Ouyang, P. *Bioresources* **2009**, *4* (3), 1147–1157.
67. Hamelinck, C. N.; van Hooijdonk, G.; Faaij, A. P. C. *Biomass Bioenergy* **2005**, *28*, 384–410.
68. Stanciu, C. *Bioresources* **2010**, *5* (3), 1436–1445.
69. Marinova, M.; Mateos-Espejel, E.; Jemaa, N.; Paris, J. *Chem. Eng. Res. Des.* **2009**, *87*, 1269–1275.
70. Marinova, M.; Mateos-Espejel, E.; Jemaa, N.; Paris, J. *Cellul. Chem. Technol.* **2010**, *44* (1−3), 21–26.
71. Zhuang, J.; Liu, Y.; Wu, Z.; Sun, Y.; Lin, L. *Bioresources* **2009**, *4* (2), 674–686.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2011.

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